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(54) Title: NOVEL HETEROARYL COMPOUNDS FOR THE MODULATION OF PROTEIN TYROSINE ENZYME RELATED CELLULAR SIGNAL TRANSDUCTION

(57) Abstract

The present invention relates to novel heteroaryl compounds and the physiologically acceptable salts and the prodrugs thereof which are expected to modulate the activity of protein tyrosine enzymes which are related to cellular signal transduction, in particular, protein tyrosine phosphatase, and therefore are expected to be useful in the prevention and treatment of disorders associated with abnormal protein tyrosine enzyme related cellular signal transduction such as cancer and diabetes.

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DESCRIPTION

NOVEL HETEROARYL COMPOUNDS FOR THE MODULATION OF PROTEIN TYROSINE ENZYME RELATED CELLULAR SIGNAL TRANSDUCTION

RELATED APPLICATIONS

The present application is related to and claims priority from provisional application serial no. 60/049,560, dated June 13, 1997, which application is incorporated as if fully set forth herein.

INTRODUCTION

The present invention relates generally to organic chemistry, biochemistry, pharmacology and medicine. More particularly, it relates to novel heteroaryl compounds and their physiologically acceptable salts and prodrugs, which modulate the activity of protein tyrosine enzymes related to cellular signal transduction and, therefore, are expected to exhibit a salutary effect against disorders associated with abnormal protein tyrosine enzyme related cellular signal transduction.

BACKGROUND OF THE INVENTION

Cellular signal transduction is a fundamental mechanism whereby external stimuli that regulate diverse cellular processes are relayed to the interior of cells. The biochemical pathways through which signals are transmitted within cells comprise a circuitry of directly or functionally connected interactive proteins. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of tyrosine residues on proteins.

The phosphorylation state of a protein may affect its conformation and/or enzymic activity as well as its cellular location. The phosphorylation state of a protein is modified through the reciprocal actions of protein tyrosine kinases (PTKS) and protein tyrosine phosphatases (PTPS) at various specific tyrosine residues.

A common mechanism by which receptors regulate cell function is through an inducible tyrosine kinase activity which is either endogenous to the receptor or is imparted by other proteins that become associated with the receptor. (Darnell et al., 1994, Science, 264:1415-1421; Heldin, 1995, Cell, 80:213-223; Pawson, 1995, Nature, 373:573-580).

Protein tyrosine kinases comprise a large family of transmembrane receptor and intracellular enzymes with multiple functional domains (Taylor et al., 1992, Ann. Rev. Cell Biol. 8:429-62). The binding of ligand allosterically transduces a signal across the cell membrane where the cytoplasmic portion of the PTKs initiates a cascade of molecular interactions that disseminate the signal throughout the cell and into the nucleus. Many receptor protein tyrosine kinase (RPTKs), such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) undergo oligomerization upon ligand binding, and the receptors self-phosphorylate (via autophosphorylation or transphosphorylation) on specific tyrosine residues in the cytoplasmic portions of the receptor (Schlessinger and Ullrich, 1992, Neuron, 9:383-91, Heldin, 1995, Cell, 80:213223). Cytoplasmic protein tyrosine kinases (CPTKs), such as Janus kinases *(e.g.,* JAK1, JAK2, TYK2) and Src kinases *(e.g.,* src, lck, fyn) are associated with receptors for cytokines *(e.g.,* IL-2, IL-3, IL-6, erythropoietin), interferons and antigens. These receptors also undergo oligomerization, and have tyrosine residues that become phosphorylated during activation, but the receptor polypeptides themselves do not possess kinase activity.

Like the PTKS, the protein tyrosine phosphatases (PTPS) comprise a family of transmembrane and cytoplasmic enzymes, possessing at least an approximately 230 amino

acid catalytic domain containing a highly conserved active site with the consensus motif [I/V]IHCXAGXXR[S/T]G. The substrates of PTPs may be PTKs which possess phosphotyrosine residues or the substrates of PTKS. (Hunter, 1989, Cell, 58:1013-16; Fischer et al., 1991, Science, 253:401-6; Saito & Streuli, 1991, Cell Growth and Differentiation, 2:59-65; Pot and Dixon, 1992, Biochem. Biophys. Acta, 1136:35-43).

Transmembrane or receptor-like PTPs (RPTPS) possess an extracellular domain, a single transmembrane domain, and one or two catalytic domains followed by a short cytoplasmic tail. The extracellular domains of these RPTPs are highly divergent, with small glycosylated segments *(e.g.,* RPTPα, RPTPε), tandem repeats of immunoglobulin-like and/or fibronectin type III domains *(e.g.,* LAR) or carbonic anhydrase like domains *(e.g.,* RPTPα, RPTPβ). These extracellular features might suggest that these RPTPs function as a receptor on the cell surface, and their enzymatic activity might be modulated by ligands. Intracellular or cytoplasmic PTPs (CPTPs), such as PTP1C and PTP1D, typically contain a single catalytic domain flanked by several types of modular conserved domains. For example, PTP1C a hemopoietic cell CPTP is characterized by two Src homology 2 (SH2) domains that recognize short peptide motifs bearing phosphotyrosine (pTyr).

In general, these modular conserved domains influence the intracellular localization of the protein. SH2-containing proteins are able to bind pTyr sites in activated receptors and cytoplasmic phosphoproteins. Another conserved domain known as SH3 binds to proteins with proline-rich regions. A third type known as the pleckstrin-homology (PH) domain has also been identified. These modular domains have been found in both CPTKs and CPTPs as well as in noncatalytic adapter molecules, such as Grbs (Growth factor Receptor Bound), which mediate protein-protein interactions between components of the signal transduction pathway (Skolnik et al., 1991, Cell, 65:83-90; Pawson, 1995, Nature, 373:573-580).

Multiprotein signaling complexes comprising receptor subunits, kinases, phosphatases and adapter molecules are assembled in subcellular compartments through the specific and dynamic interactions between these domains with their binding motifs. Such signaling complexes integrate the extracellular signal with the ligand-bound receptor and relay the signal to other downstream signaling proteins or complexes in other locations inside the cell or in the nucleus (Koch et al., 1991, Science, 252:668-674; Pawson, 1994, Nature, 373:573-580; Mauro et al., 1994, *Trends Biochem. Sci.*, 19:151-155; Cohen et al., 1995, Cell, 80:237-248).

The levels of tyrosine phosphorylation required for normal cell growth and differentiation at any time are achieved through the coordinated action of PTKs and PTPS. Depending on the cellular context, these two types of enzymes may either antagonize or cooperate with each other during signal transduction. An imbalance between these enzymes may impair normal cell functions leading to metabolic disorders and cellular transformation.

For example, insulin binding to the insulin receptor, which is a PTK, triggers a variety of metabolic and growth promoting effects such as glucose transport, biosynthesis of glycogen and fats, DNA synthesis, cell division and differentiation. Diabetes mellitus, which is characterized by insufficient or a lack of insulin signal transduction, can be caused by any abnormality at any step along the insulin signaling pathway. (Olefsky, 1988, "Cecil Textbook of Medicine," 18th Ed., 2:1360-81).

It is also well known, for example, that the overexpression of PTKS, such as HER2, can play a decisive role in the development of cancer (Slamon et al., 1987, Science, 235:77-82) and that antibodies capable of blocking the activity of this enzyme can abrogate tumor growth (Drebin et al., 1988, Oncogene, 2:387-394). Blocking the signal transduction capability of tyrosine kinases such as Flk-1 and the PDGF receptor have been shown to block tumor growth in animal models (Millauer et al., 1994, Nature, 367:577; Ueno et al., Science, 252:844-848).

Relatively less is known with respect to the direct role of tyrosine phosphatases in signal transduction; PTPs may play a role in human diseases. For example, ectopic expression of RPTPα produces a transformed phenotype in embryonic fibroblasts (Zheng et al., Nature, 359:336-339), and overexpression of RPTPα in embryonal carcinoma cells causes the cells to differentiate into a cell type with a neuronal phenotype (den Hertog, et al., EMBO Journal, 12:3789-3798). The gene for human RPTPγ has been localized to chromosome 3p21 which is a segment frequently altered in renal and small lung carcinoma. Mutations may occur in the extracellular segment of RPTPγ which renders the RPTP no longer responsive to external signals (LaForgia et al., Wary et al., 1993, Cancer Res., 52:478-482). Mutations in the gene encoding PTP1C (also known as HCP or SHP) are the cause of the moth-eaten phenotype in mice which suffer from severe immunodeficiency, and systemic autoimmune disease accompanied by hyperproliferation of macrophages (Schultz et al., 1993, Cell, 73:1445-1454). PTP1D (also known as Syp or PTP2C) has been shown to bind through SH2 domains to sites of phosphorylation in PDGFR, EGFR and insulin receptor substrate 1 (IRS-1). Reducing the activity of PTP1D by microinjection of anti-PTP1D antibody has been shown to block insulin or EGF-induced mitogenesis (Xiao et al., 1994, J. Biol. Chem., 269:21244-21248).

It has been reported that some of the biological effects of insulin can be mimicked by vanadium salts such as vanadates and pervanadates. Vanadates and pervanadates are known to be non-specific phosphatase inhibitors. However, this class of compounds is toxic because each compound contains a heavy metal (U.S. Patent No. 5,155,031; Fantus et al., 1989, Biochem., 28:8864-71; Swarup et al., 1982, Biochem. Biophys. Res. Commun., 107:1104-9).

SUMMARY OF THE INVENTION

The present invention relates generally to novel heteroaryl compounds which modulate the activity of protein tyrosine enzymes which are related to cellular signal transduction; namely, protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). In particular, the compounds of this invention are expected to modulate protein tyrosine phosphatase activity. In addition, the present invention relates to the preparation and use of pharmaceutical compositions of the disclosed compounds and their physiologically acceptable salts and prodrugs for the treatment or prevention of disorders associated with abnormal protein tyrosine enzyme related cellular signal tranduction including, but not limited to, cancer and diabetes.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention wherein it is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is not beneficial, but then it is metabolically hydrolyzed to the carboxylic acid once inside the cell where water solubility is beneficial.

As used herein, an "ester" is a C-carboxy group, as defined herein, wherein R" is any of the listed groups other than hydrogen.

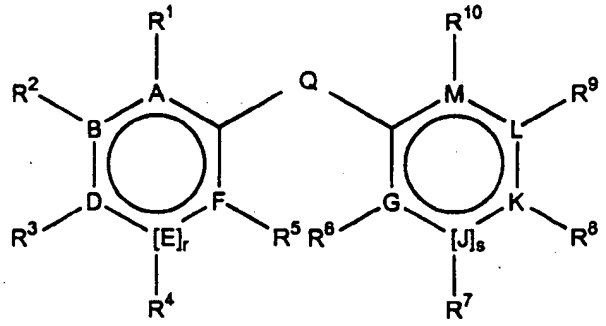
As used herein, a "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

THE COMPOUNDS

General structural features.

In one aspect this invention relates to heteroaryl compounds having the general chemical structure shown in Formula 1:



1

In Formula 1, r and s are independently 0 or 1.

When r or s is 1 then A, B, D, E, F, G, J, K, L, and M are independently selected from the group consisting of carbon and nitrogen, it being understood that the six-member nitrogen heteroaryl rings so formed are those known in the chemical arts; it being further understood that when A, B, D, E, F, G, J, K, L or M is nitrogen, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 or R^{10}, respectively, do not exist.

At least one of A, B, D, E or F and at least one of G, J, K, L and M must be nitrogen.

When r or s is 0 then A, B, D, and F or G, K, L and M, respectively, are independently selected from the group consisting of carbon, nitrogen, oxygen and sulfur, it being understood that the five-member heteroaryl rings so formed are those known in the chemical arts; it being further understood that when A, B, D, F, G, K, L or M is oxygen or sulfur or A, B, D, F, G, K, L or M is nitrogen and that nitrogen is participating in a heteroaryl ring double bond, R^1, R^2, R^3, R^5, R^6, R^8, R^9 or R^{10} do not exist.

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisiting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, alkoxy, aryloxy, thioalkoxy, thioaryloxy, heteroaryloxy, heteroalicycloxy, sulfinyl, sulfonyl, S-sulfonamido, N-Sulfonamido, trihalomethanecarbonyl, trihalomethanesulfonyl, carbonyl, C-carboxy, O-carboxy, C-amido, C-thioamido, N-amido, hydrazino, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, phosphonyl, N-thiocarbamyl, guanyl, guanidino, ureido, amino, trihalomethane sulfonamido, and $-NR^{11}R^{12}$.

R^{11} and R^{12} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, carbonyl, C-carboxy, sulfonyl, trihalomethanesulfonyl, trihalomethanecarbonyl and, combined, a five- or six-member heteroalicyclic ring.

When r or s is 0 and A, B, D, or F; or G, K, L or M, respectively, is a nitrogen atom which is not participating in a heteroaryl ring double bond, then R^1, R^2, R^3, R^5, R^6, R^8, R^9 and R^{10} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, trihalomethanecarbonyl, sulfonyl, trihalomethane- sulfonyl, cyano, C-carboxy, O-carboxy, C-amido, C-thioamido and guanyl;

Q is selected from the group consisting of oxygen, sulfur, sulfinyl, sulfonyl and $-NR^{13}$.

R^{13} is selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl,

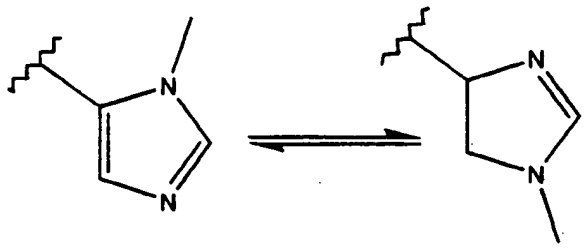
alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, cyano, trihalomethanecarbonyl, sulfonyl, trihalomethanesulfonyl, C-carboxy, O-carboxy, C-amido, C-thioamido and guanyl.

Any two adjacent R groups may combine to form an additional aryl, cycloalkyl, heteroaryl or heteroalicyclic ring fused to the ring initially bearing those R groups.

When r is 0, A is sulfur, F is nitrogen, Q is sulfur and R^2 is nitro, then G, J, K, L, M, R^6, R^7, R^8, R^9 and R^{10} are selected so as to afford the compounds of Table 1.

Physiologically acceptable salts and prodrugs of the compounds disclosed herein are within the scope of this invention.

As used herein, the phrase "participating in a heteroaryl ring double bond", with regard to A, B, D, F, G, K, L and M when they are nitrogen atoms, refers to the formal double bonds in the two tautomeric structures which comprise five-member ring heteroaryl groups:



An "alkyl" group refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc. up to and including 20 carbon atoms). More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more individually selected from trihaloalkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, heteroaryloxy, heteroalicycloxy, thiohydroxy, thioalkoxy,

thioaryloxy, thioheteroaryloxy, thioheteroalicycloxy, cyano, halo, nitro, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, C-thioamido, N-amido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, sulfonamido, trihalomethane-sulfonamido, trihalomethanesulfonyl, silyl, guanyl, guanidino, ureido, phosphonyl, amino and $-NR^{11}R^{12}$ wherein R^{11} and R^{12} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, carbonyl, C-carboxy, sulfonyl, trihalomethanesulfonyl, trihalomethanecarbonyl, and, combined, a five- or six-member heteroalicyclic ring.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more individually selected from alkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, heteroaryloxy, heteroalicycloxy, thiohydroxy, thioalkoxy, thioaryloxy, thioheteroaryloxy, thioheteroalicycloxy, cyano, halo, nitro, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, C-thioamido, N-amido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, sulfonamido, trihalo- methanesulfonamido, trihalomethanesulfonyl, silyl, guanyl, guanidino, ureido, phosphonyl, amino and $-NR^{11}R^{12}$ with R^{11} and R^{12} as defined above.

An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more selected from alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, heteroaryloxy, heteroalicycloxy, thiohydroxy, thioalkoxy, thioaryloxy, thioheteroaryloxy, thioheteroalicycloxy, cyano, halo, nitro, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, C-thioamido, N-amido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, sulfonamido, trihalomethanesulfonamido, trihalomethane- sulfonyl, silyl, guanyl, guanidino, ureido, phosphonyl, amino and $-NR^{11}R^{12}$ with R^{11} and R^{12} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more selected from alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, heteroaryloxy, heteroalicycloxy, thiohydroxy, thioalkoxy, thioaryloxy, thioheteroaryloxy, thioheteroalicycloxy, cyano, halo, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, C-thioamido, N-amido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, sulfonamido, nitro, trihalomethanesulfonamido, trihalomethanesulfonyl, silyl, guanyl, guanidino, ureido, phosphonyl, amino and $-NR^{11}R^{12}$ with R^{11} and R^{12} as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more selected from alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, heteroaryloxy, heteroalicycloxy, thiohydroxy, thioalkoxy, thioaryloxy, thioheteroaryloxy, thioheteroalicycloxy, cyano, halo, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, C-thioamido, N-amido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, sulfonamido, nitro, trihalomethanesulfonamido, trihalomethanesulfonyl, silyl, guanyl, guanidino, ureido, phosphonyl, amino and $-NR^{11}R^{12}$ with R^{11} and R^{12} as defined above.

A "hydroxy" group refers to an -OH group.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "heteroaryloxy" group refers to a heteroaryl-O- group with heteroaryl as defined herein.

A "heteroalicycloxy" group refers to a heteroalicyclic-O- group with heteroalicyclic as defined herein.

A "thiohydroxy" group refers to an -SH group.

A "thioalkoxy" group refers to both an S-alkyl and an -S-cycloalkyl group, as defined herein.

A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

A "thioheteroaryloxy" group refers to a heteroaryl-S- group with heteroaryl as defined herein.

A "thioheteroalicycloxy" group refers to a heteroalicyclic-S- group with heteroalicyclic as defined herein.

A "carbonyl" group refers to a -C(=O)-R" group, where R" is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) and heteroalicyclic (bonded through a ring carbon), as each is defined herein.

An "aldehyde" group refers to a carbonyl group where R" is hydrogen.

A "thiocarbonyl" group refers to a -C(=S)-R" group, with R" as defined herein.

A "keto" group refers to a –CC(=O)C- group wherein the carbon on either or both sides of the C=O may be alkyl, cycloalkyl, aryl or a carbon of a heteroaryl or heteroaliacyclic group.

A "trihalomethanecarbonyl" group refers to a $X_3CC(=O)$- group with X as defined herein.

A "C-carboxy" group refers to a -C(=O)O-R" groups, with R" as defined herein.

An "O-carboxy" group refers to a R"C(=O)O- group, with R" as defined herein.

A "carboxylic acid" group refers to a C-carboxy group in which R" is hydrogen.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

A "trihalomethanecarbonyl" group refers to an $X_3CC(=O)$- group with X as defined above.

A "trihalomethanesulfonyl" group refers to an $X_3CS(=O)_2$- groups with X as defined above.

A "trihalomethanesulfonamido" group refers to a $X_3CS(=O)_2NR^{13}$- group with X and R^{13} as defined herein.

A "sulfinyl" group refers to a -S(=O)-R" group, with R" as defined herein and, in addition, as a bond only; i.e., -S(O)-.

A "sulfonyl" group refers to a -$S(=O)_2$R" group, with R" as defined herein and, in addition as a bond only; i.e., -$S(O)_2$-.

An "S-sulfonamido" group refers to a -$S(=O)_2NR^{11}R^{12}$, with R^{11} and R^{12} as defined herein.

An "N-Sulfonamido" group refers to a $R^{11}S(=O)_2NR^{12}$- group, with R^{11} and R^{12} as defined herein.

An "O-carbamyl" group refers to a -$OC(=O)NR^{11}R^{12}$ group with R^{11} and R^{12} as defined herein.

An "N-carbamyl" group refers to a $R^{11}OC(=O)NR^{12}$ group, with R^{11} and R^{12} as defined herein.

An "O-thiocarbamyl" group refers to a -$OC(=S)NR^{11}R^{12}$ group with R^{11} and R^{12} as defined herein.

An "N-thiocarbamyl" group refers to a $R^{11}OC(=S)NR^{12}$- group, with R^{11} and R^{12} as defined herein.

An "amino" group refers to an -NH_2 group.

A "C-amido" group refers to a -$C(=O)NR^{11}R^{12}$ group with R^{11} and R^{12} as defined herein.

A "C-thioamido" group refers to a -$C(=S)NR^{11}R^{12}$ group, with R^{11} and R^{12} as defined herein.

An "N-amido" group refers to a $R^{11}C(=O)NR^{12}$- group, with R^{11} and R^{12} as defined herein.

A "ureido" group refers to a -$NR^{11}C(=O)NR^{12}R^{14}$ group, with R^{11} and R^{12} as defined herein and R^{14} defined the same as R^{11} and R^{12}.

A "guanidino" group refers to a $-R^{11}NC(=N)NR^{12}R^{14}$ group, with R^{11}, R^{12} and R^{14} as defined herein.

A "guanyl" group refers to a $R^{11}R^{12}NC(=N)-$ group, with R^{11} and R^{12} as defined herein.

A "cyano" group refers to a $-C\equiv N$ group.

A "silyl" group refers to a $-Si(R")_3$, with R" as defined herein.

A "phosphonyl" group refers to a $P(=O)(OR^{11})_2$ with R^{11} as defined herein.

A "hydrazino" group refers to a $-NR^{11}NR^{12}R^{14}$ group with R^{11}, R^{12} and R^{14} as defined herein.

Preferred Structural Features.

Preferred structural features of this invention are those in which r and s are 0 and Q is sulfur.

Further preferred structural features are those in which r is 1 and s is 0.

Other preferred structural features are those in which r is 0, A is sulfur, F is nitrogen, Q is sulfur and R^2 is nitro.

The compounds in Table 1 provide additional preferred structural features of this invention.

THE BIOCHEMISTRY

The present invention is directed to the use of compounds capable of modulating or regulating signal transduction in normal or diseased cells. The present invention is also directed to the use of compounds capable of inhibiting the activity of protein tyrosine enzymes, in particular protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), to modulate or trigger signal transduction. The invention is further directed to the regulation of cellular processes that are controlled by signal transduction through the

inhibition of the activity of PTKs and PTPs by the compounds. The invention further provides for the use of such compounds in the treatment of a subject having a disorder caused by dysfunctional signal transduction.

In one embodiment of the invention, the compounds of the invention are capable of inhibiting the activity of protein tyrosine phosphatases, that are transmembrane or intracellular, and that may have one or more characteristic catalytic domains. The amino acid sequences of the PTPs in the catalytic domains may include but are not limited to [I/V]HCXAGXXR(S/T]G (single-letter amino acid code; X is any amino acid). In addition, the PTPs may possess one or more modular conserved domains, which include but are not limited to, SH2, SH3 and PH domains. In a specific embodiment of the invention, the compounds of the invention can be used to inhibit the phosphatase activity of PTP1B (Charbonneau, et al., 1989, Proc. Natl. Acad. Sci., USA, 86: 5252-5256), T-cell PTP (Cool, et al., 1989, Proc. Natl. Acad. Sci., USA, 86: 52575261, PTP1C (Shen, et al., 1991, Nature, 352: 736-739), PTP1D (Vogel, et al., 1993, Science, 259: 1611-1614), RPTPα, RPTPβ, RPTPγ (Kaplan, et al., 1990, Proc. Natl. Acad. Sci., USA, 87: 70007004), RPTPα (Yan, et al., 1993, J. Biol. Chem., 268: 24880-24886), RPTPκ (Jiang , et al., 1993, Mol. Cell Biol., 13: 2942-2951) and CD45 (Charbonneau, et al., 1988, Proc. Natl. Acad. Sci., USA, 85: 7182-7186). The PTKs and PTPs preferred in the invention are of human origin. Inhibition of phosphatase activity that is substantially specific to a PTP or a set of PTPs in a signaling pathway is preferred. While the inhibition of phosphatase activity is believed to be the mechanism of action of the compounds of the present invention with respect to their ability to modulate and/or regulate signal transduction, additional mechanisms have not been ruled out.

The term "signal transduction" as used herein is not limited to transmembrane signaling, and includes the multiple pathways that branch off throughout the cell and into the nucleus. Such signaling pathways may include but are not limited to the Ras pathway

(Schlessinger, 1994, Curr. Opin. Genet. Dev., 4:25-30), the JAK/STAT pathways (Sadowski, et al., 1994, Science, 261:1739-1744), the phosphoinositide 3-kinase pathway and the phospholipase C-γ pathway. As used herein, the term "modulation" or "modulating" shall mean upregulation or downregulation of a signaling pathway. Cellular processes under the control of signal transduction may include, but are not limited to, transcription of specific genes; normal cellular functions, such as metabolism, proliferation, differentiation, adhesion, apoptosis and survival; as well as abnormal processes, such as transformation, blocking of differentiation and metastasis.

A signal may be triggered by the binding of a ligand to its receptor on the cell surface, and the signal is transduced and propagated by the phosphorylation or dephosphorylation of specific tyrosine residues on various substrates inside the cell. The specific interactions between the PTKs, PTPs and their substrates may involve the formation of a transient or stable multimolecular complex on the inner face of the plasma membrane or in other subcellular compartments including the nucleus. A substrate may contain one or more tyrosine residues that are phosphorylated or dephosphorylated by PTKs or PTPs in the signaling pathway. Such substrates may include the receptor and its subunits, molecules associated with or recruited to the receptor such as cytoplasmic kinases, cytoplasmic phosphatases, adapter molecules, cytoskeletal proteins and transcription factors. The term receptor as used herein may include, but is not limited to, insulin receptor, members of the insulin-like growth factor receptor family, epidermal growth factor receptor family, fibroblast growth factor receptor family, hepatocyte growth factor receptor family, vascular endothelial growth factor receptor family, neurotrophin receptor (trk) family, the T-cell receptor, the B cell receptor and members of the Type I-IV cytokine receptor families (Heldin, 1995, Cell, 80: 213-223; Taniguchi, 1995, Science, 268: 251-255). Adapter molecules that are substrates may include the Grb proteins, IRS-1, Zap-70 and Shc (Pawson, et al., 1995, Nature, 373:

573-580). Cytoskeletal proteins such as actin and transcription factors such as the STAT proteins (Ihle, et al., Trends Biochem. Sci., 19:222-227) may also serve as substrates. As used herein, the term ligand is synonymous with extracellular signaling molecules, and includes but is not limited to growth factors such as insulin, EGF, PDGF, fibroblast growth factors, vascular endothelial growth factor, and neurotrophins; and cytokines such as growth hormone, erythropoietin, tumor necrosis factor, interleukins and interferons. The term ligand is not limited to soluble molecules, and includes, for example, extracellular matrix proteins, cell adhesion molecules as well as antigenic peptides associated with the major histocompatibility complex proteins on the surface of an antigen-presenting cell.

In one embodiment of the invention, the compounds of the invention can be used to trigger or upregulate signal transduction in cells so that the effect of ligand binding to a receptor is enhanced, or mimicked if the ligand is not present. The compounds exert the effect by inhibiting or diminishing the activity of a phosphatase in the signaling pathway which normally acts negatively toward signaling. One mechanism by which PTPs normally downregulate signal transduction involves the dephosphorylation of specific phosphotyrosine residues (pTyr) on PTKs and their substrates since many PTKs require phosphorylation of some of its own tyrosine residues in order to become optimally active in the signaling pathway. The compounds of the invention can be used to prevent the dephosphorylation of pTyr residues on receptors or their subunits which normally becomes phosphorylated upon ligand binding, thereby enhancing the extent and duration of PTK phosphorylation. The compounds of the invention can also be used to prevent the dephosphorylation of PTKs in which the tyrosine residues become autophosphorylated or transphosphorylated due to its basal activity. In these PTKS, a signal may be triggered by the compounds of the invention in the absence of ligand binding since the basal activity of PTKs is sufficient to promote a signal if constitutive PTP activity is inhibited or diminished by the compounds.

A preferred embodiment of the invention is directed to a method of triggering, enhancing or sustaining insulin receptor signal transduction by inhibiting the constitutive, dephosphorylation of the pTyr sites on the activated insulin receptor. This would allow the insulin receptor to remain phosphorylated, thus enhancing or sustaining the insulin signal. Furthermore, since it has been shown that insulin receptor is phosphorylated at a low level even in the absence of insulin (Goldstein, 1992, J. Cell Biol., 48:33-42), the compounds of the invention can be used to trigger a signal, even in the absence of insulin, by allowing the tyrosine residues on the receptor to become self-phosphorylated.

Another mechanism by which PTPs may exert a negative effect on signaling is through the dephosphorylation of specific pTyr sites to which SH2-containing molecules bind during signaling. The absence of such pTyr sites would prevent the recruitment of SH2-containing molecules to specific subcellular compartments to form multiprotein signaling complexes, thereby, preventing the further propagation of the signal. Thus, the compounds of the invention can be used to upregulate or prolong signal transduction by preventing the dephosphorylation of pTyr sites on substrate proteins that normally serve as binding sites for SH2-containing proteins which promote signaling. In another embodiment of the invention, the compounds of the invention may be used to prevent the dephosphorylation of specific pTyr residues on any substrate, which pTyr residues are essential to the transmissions or propagation of the signal. Furthermore, the compounds of the invention may be used to prevent the dephosphorylation of specific pTyr residues on any substrate, which pTyr residues are inhibitory to signal transduction.

The compounds of the invention can also be used to suppress or downregulate signal transduction in cells so that the effect of ligand binding to a receptor is abolished or attenuated. The compounds can inhibit a phosphatase in a signaling pathway which normally acts positively toward signaling. For example, PTPs promote signaling through the activation

of members of the Src family of PTKs. Src family PTKs have an inhibitory site of phosphorylation in their carboxy termini which by dephosphorylation activates kinase activity. Thus the compounds of the invention can be used to prevent the dephosphorylation of the inhibitory pTyr in the carboxy termini of kinases which function normally to promote signal transductions. Src family PTKs may include Src, Fyn, Lck, Lyn, Blk, Hck, Fgr and Yrk. Other kinases which may be similarly regulated by a phosphatase may include Fak and Csk (Taniguchi, 1995, Science, 268: 251-255).

PHARMACOLOGICAL COMPOSITIONS AND THERAPEUTIC APPLICATIONS

As used herein, "pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the compound and which are obtained by reaction with acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

In addition to the above compounds and their pharmaceutically acceptable salts, the present invention is further directed, where applicable, to solvated as well as unsolvated forms of the compounds *(e.g.,* hydrated forms) having the ability to regulate and/or modulate phosphatase activity.

The compounds described above may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Suitable processes are illustrated by the representative examples provided, infra. Necessary starting materials may be obtained by standard procedures of organic chemistry.

Pharmaceutical Formulations And Routes Of Administration

A compound of this invention can be administered to a human patient as such or in pharmaceutical compositions in which a therapeutically effective dose is mixed with suitable

carriers or excipient(s) at doses to treat or ameliorate a variety of disorders, including solid cell tumor growth, including Kaposi's sarcoma, glioblastoma, and melanoma and ovarian, lung, mammary, prostate, pancreatic, colon and epidermoid carcinoma, diabetes, diabetic retinopathy, hemangioma and rheumatoid arthritis. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms of uncontrolled vasculogenesis and angiogenesis. Techniques for formulation and administration of the compounds such as those of this invention may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

The formulations of the present invention normally will consist of at least one compound of formula I mixed with a carrier, or diluted by a carrier, or enclosed or encapsulated by an ingestible carrier in the form of a capsule, sachet, cachet, paper or other container or by a disposable container such as an ampoule. A carrier or diluent may be a solid, semi-solid or liquid material, which serves as a vehicle, excipient or medium for the active therapeutic substance.

Some examples of the diluents or carriers which may be employed in the pharmaceutical compositions of the present invention are lactose, dextrose, sucrose, sorbitol, mannitol, propylene glycol, liquid paraffin, white soft paraffin, kaolin, microcrystalline cellulose, calcium silicate, silica polyvinylpyrrolidone, cetostearyl alcohol, starch, gum acacia, calcium phosphate, cocoa butter, oil of theobroma, arachis oil, alginates, tragacanth, gelatin, syrup B.P., methyl cellulose, polyoxyethylene sorbitan monolaurate, ethyl lactate and propylhydroxybenzoate, sorbitan trioleate, sorbitan sesquioleate and oleyl alcohol.

Routes Of Administration

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharamacological composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or

treatment of a disorder associated with an abnormal protein tyrosine enzyme related cellular signal transduction.

As used herein, a "disorder associated with an abnormal protein tyrosine enzyme related cellular signal transduction" refers to a condition characterized by inappropriate; i.e., under or, more commonly, over, catalytic activity on the part of a protein tyrosine enzyme. Inappropriate catalytic activity can arise as the result of either: (1) protein tyrosine enzyme expression in cells which normally do not express protein tyrosine enzymes; (2) increased protein tyrosine enzyme expression leading to unwanted cell proliferation, differentiation and/or growth; or, (3) decreased protein tyrosine enzyme expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of protein tyrosine enzymes refers to either amplification of the gene encoding a particular protein tyrosine enzyme or production of a level of protein tyrosine enzyme activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the protein tyrosine enzyme increases, the severity of one or more of the symptoms of the cellular disorder increases). Underactivity is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the protein tyrosine enzyme decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from in the first place acquiring a disorder associated with abnormal protein tyrosine enzyme related cellular signal transduction.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating the abnormal protein tyrosine enzyme related cellular signal transduction disorder and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukariotic cell or as complex as a mammal, including a human being.

Suitable routes of administration include, without limitation, oral, rectal, transmucosal, or intestinal administration; intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections; transdermal, topical and vaginal application, and the like. Dosage forms include but are not limited to tablets, troches, dispersions, suspensions, suppositories, solutions, capsules, creams, patches, minipumps and the like.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation

The pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, for example and without limitation by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can, for instance, be prepared by adding a compound of this invention to a solid excipient, optionally grinding the resulting mixture and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner. For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds of this invention may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions

of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives such as, for example, a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:05W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This

co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied, for example, other low toxicity nonpolar surfactants may be used instead of polysorbate 80®; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.*, polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Numerous sustained release products are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means and/or delivery devices including Alzet® osmotic pumps which are available from Alza Corporation. Suitable

delivery devices are described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,944,064 and 4,008,719, the disclosures of which are incorporated in their entirety by reference herein.

Many of the phosphatase modulating compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

Dosage

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve its intended purpose. The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor; (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis; (3) inhibiting to some extent (that is slowing to some extent, preferably stopping) tumor growth; and/or, (4) relieving to some extent (or preferably eliminating) one or more symptoms associated with the cancer. Determination of the therapeutically effective amount of a compound of this invention is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

The therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC50 as determined in cell culture *(i.e.,* the

concentration of the test compound which achieves a half-maximal inhibition of the PTP activity). Such information can be used to more accurately determine useful doses in humans.

Thus, a therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms in or a prolonged survival of a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The ratio of toxic does to therapeutic effective, LD50/ ED50, is the therapeutic index. Compounds which exhibit high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. A dosage preferably lies within a range of circulating concentrations that include the ED5O and exhibits little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1, p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the tyrosine enzyme modulating effects, known as the minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vitro data; for example, without limitation, the concentration necessary to achieve a 50-90% inhibition of the tyrosine enzyme using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90%, preferably between 30-90% and most preferably between 50-90% of the time.

Usual patient dosages for systemic administration range from 1 to 2000 mg/day, commonly from 1 to 250 mg/day, and typically from 10 to 150 mg/day. Stated in terms of patient body weight, usual dosages range from 0.02 to 25 mg/kg/day, commonly from 0.02 to 3 mg/kg/day, typically from 0.2 to 1.5 mg/kg/day. Stated in terms of patient body surface areas, usual dosages range from 0.5 to 1200 mg/m^2/day, commonly from 0.5 to 150 Mg/m^2/day, typically from 5 to 100 Mg/ m^2/day. Usual average plasma levels should be maintained within 50 to 5000 μg/ml, commonly 50 to 1000 μg/ml, and typically 100 to 500 μg/ml.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of a particular composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

Desirable blood levels may be maintained by a continuous infusion of the compound; plasma level can be monitored by HPLC. It should be noted that the attending physician would know how and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response is not adequate and toxicity os not a problem.

The size of a prophylactic or therapeutic dose of a compound in the acute or chronic management of disease will vary with the severity of the condition to be treated and the route

of administration. Again, it should be noted that the clinician or physician would know when to interrupt and/or adjust the treatment dose due to toxicity or bone marrow, liver or kidney dysfunctions. The dose, and perhaps the dosage frequency, will also vary according to the age, body weight, and response of the individual patient. In general, as discussed above, the total daily dose ranges for the compounds for the majority of the disorders described herein, is from about 0.02 to about 25 mg/kg patient. Preferably, a daily dose range should be between about 0.02 to about 3 mg/kg, while most preferably a daily dose range should be between about 0.2 to about 1.5 mg/kg per day. It is further recommended that infants, children, and patients over 65 years, and those with impaired renal, or hepatic function, initially receive low doses and that they be titrated based on individual clinical response(s) and blood level(s). It may be necessary to use dosages outside the above ranges in some cases; situations requiring such a decision will be apparent to those of ordinary skill in the art.

Packaging

The compositions may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of the labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated

on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

Methods Of Treatment

Any compound of the invention which inhibits or diminishes protein tyrosine enzyme activity in a signaling pathway may be used in the therapeutic methods of the invention. In a preferred embodiment, the activity of the compound is sufficiently specific for the paticular protein tyrosine enzyme pathway so that the compound does not interfere with the function of other enzymatic activity, including other tyrosine enzyme activity, in the cell.

The compounds and pharmaceutical compositions of the invention can be used for treating diabetes mellitus. The pathogenesis of diabetes generally involves insufficient or a total lack of insulin signal transduction. The paucity or absence of the insulin signal may be caused by a variety of factors such as a lack of insulin, loss of binding affinity, defective receptor or under expression of receptor. Insulin receptor activity can be modulated by inhibiting tyrosine phosphatases in the signaling using the compounds of the invention. Unlike currently available treatment modalities that are based on the insulin receptor, the insulin signal may be restored or stimulated in cells through the inhibition of dephosphorylating activity, even in the absence of insulin. The example of diabetes mellitus illustrates the principles of therapeutic applications of the compounds of this invention which may be applied to other disorders that implicate signal transduction by tyrosine enzymes, in particular, phosphotyrosine phosphatases. The compounds and pharmaceutical compositions of the invention may be used to treat immune disorders in which cytokine signal transduction is deficient. Cytokines play a crucial role in hemopoiesis as well as coordinating immune and inflammatory responses. The compounds may be used to replace or enhance the activity of a cytokine in signaling the differentiation and proliferation of hemopoietic cells, as well as B and T cells in response to antigenic stimulation, and thus be useful for treating anemia and

immunodeficiency. The compounds may also be used as an antiinflammatory agent to treat disorders such as rheumatoid arthritis. The compounds may also be therapeutically useful in treating neurodegenerative diseases by stimulating the growth and differentiation of neuronal cells which is regulated by neurotrophin-mediated signal transduction.

In another embodiment of the invention, the compounds and pharmaceutical compositions of the invention may be used to treat cancer, such as glioma, melanoma, Kaposi's sarcoma, hemangioma and ovarian, breast, lung, pancreatic, liver, prostate, colon and epidermoid cancer, in which the malignant cells proliferate and/or metastasize as a result of uncontrolled signal transduction mediated by growth factors. For example, over expression of a PTK, such as HER2 has been shown to correlate with the aberrant growth characteristics of tumor cells. Vasculogenesis and/or angiogenesis that facilitates tumor growth may also be inhibited by the compounds of this invention. The compounds may modulate signal transduction in these tumor cells so that normal growth characteristics are restored. The compounds may also be useful in treating psoriasis which is caused by excessive epidermal growth factor mediated signal transduction.

SYNTHESIS

The compounds of the present invention as well as the starting materials may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of this invention are available and that the following examples are in no way to be considered limiting in any manner whatsoever with regard to preparation of the compounds of this invention.

Example 1. 3-(5-nitrothiazol-2-yl)mercaptol-5-phenyl-1,2,4-triazole (Compound 1)

The starting material 2-brono-5- nitrothiazol was prepared by treating 2-amino-5-

nitrothiazol (Aldrich) with sodium nitrite and hydrogen bromide (Fr. Demande 2,015,4 34, 1970). 3-Phenyl-1,2,4-triazole-5-thione (E. Hogarth, J. *Chem. Soc.* (1949) 1163) was prepared by first reacting benzoyl chloride with thiosemicarbazide in pyridine at 0 °C to give benzoyl thiosemicarbazide. Benzoyl thiosemicarbazide was treated with potassium hydroxide in ethanol to give 3phenyl-1,2,4-triazole-5-thione. 3-Phenyl-1,2,4-triazole-5thione (1.77 g) was then dissolved in 50 mL of methanol and treated with 0.57 g of 95% sodium methoxide, and then with 2-bromo-5-nitrothiazole (2.09 g). The mixture was stirred at room temperature for 2 hours and the precipitated sodium bromide was removed by filtration. The methanol was evaporated and the product crystallized from ethanol and watre to give 1.5 g of 3-[(5-nitrothiazol-2-yl)mercapto]-5-phenyl 1,2,4-triazole, a white solid, MP 155-157°C.

Example 2. 2-[(5-nitro-thiazol-2-yl)mercapto]-5-t-butyl-1,2,4-triazole

The title compound was prepared in the manner described in Example 1. Substituting pivaloyl chloride for the benzoyl chloride in Example 1 gave pivaloyl thiosemicarbazide and then 3-t-butyl-1,2,4-triazole-5-thione. Reaction of 1.79 g of the sodium salt of the thione with 2.09 g of 2-bromo-5-nitrothiazole as in Example 1 yielded 1 g of 2-[(5-nitro-thiazol-2-yl)- mercapto]-5-t-butyl-1,2,4-triazole, a yellow solid, MP 219-221° C.

Example 3. 3-[(5-nitrothiazol-2-yl)mercapto]-5-(thien-2-yl)-1,2,4-triazole

The title compound was prepared in the manner described in Example 1. Substituting the acid chloride of thiophene-2-carboxylic acid (prepared from the acid and oxalyl chloride) for the benzoyl chloride in Example 1 gave the thiosemicarbazide of thiophene-2-carboxylic acid and then 3-(thien-2-yl)-1,2,4-triazole-5-thione. Reaction of 1.73 g of the sodium salt of the thione with 2.09 g of 2-bromo-5-nitrothiazole as in Example 1 yielded 1 g of 3-[(5-nitrothiazol- 2-yl)mercapto]-5-(thien-2-yl)-1,2,4-triazole, an orange solid, MP 179-181° C.

Example 4. 3-(4-chlorophenyl)-5-[(5-nitrothiazol-2yl)mercapto]-1,2,4-triazole

The title compound was prepared in the manner described in Example 1. Substituting 4-chlorobenzoyl chloride for the benzoyl chloride in Example 1 gave 4-chlorobenzoyl thiosemicarbazide and then 3-(4-chlorophenyl)-1,2,4-triazole-5-thione. Reaction of 2.34 g of the sodium salt of 3-(4-chlorophenyl)-1,2,4-triazole-5-thione with 2.09 g of 2-bromo--5-nitrothiazole as in Example 1 yielded 1.5 g of 3-(4--chlorophenyl)-5-[(5-nitrothiazol-2-yl)mercapto[-1,2,4triazole, a light brown solid, MP 181-184°C.

Example 5. 3-hydroxy-5-[(5-nitrothiazol-2 yl)mercapto]-4-phenyl-1,2,4-triazole

The title compound was prepared by the general method described by Potts, K.T., 1961, Chem. Rev., 61:87. 4-Phenyl-3-thiosemicarbazide (4.18g) (Aldrich) was dissolved in 50 mL of pyridine and treated with 2.71 g of ethyl chloroformate at O°C. The reaction was stirred for 2 hours and then refluxed for 18 hours. Evaporation of the solvent and trituration with water gave 2.5 g of 3-hydroxy-5-mercapto-4-phenyl-1,2,4-triazole. 3-Hydroxy-5-mercapto-4-phenyl- 1,2,4-triazole (1.93 g) was stirred in 10 mL of ethanol with 1.1 equivalent of potassium carbonate in 10 mL ethanol for one hour and then reacted with 2.09 g of 2-bromo-5-nitrothiazole as in Example 1. Crystallization from ethanol and water gave 0.6 g of 3-hydroxy-5-[(5-nitro- thiazol-2-yl)mercapto]-4-phenyl-1,2,4-triazole, a dark yellow solid, M.P. 188-190°C.

Example 6. 4-Cyclohexyl-3-hydroxy-5-[(5-nitrothiazol-2-yl)mercapto]-1,2,4-triazole

The title compound was prepared in a manner similar to that described in Example 5. Cyclohexyl isothiocyanate (3.53 g) in 10 mL of acetonitrile was added to hydrazine (0.8 g) in

20 mL of acetonitrile over a period of 30 minutes. The reaction was stirred for two additional hours and evaporated to dryness to give 4.4 g of 4-cyclohexylcarbonyl-3-thiosemicarbazide. 4-Cyclohexylcarbonyl-3-thiosemicarbazide (2.02 g) was treated as in Example 5 with ethyl chloroformate (1.08 g). The reaction product 3-hydroxy-5-mercaptol-4-cyclohexyl-1,2,4-triazole (1.0 g) was reacted with 1.05 g of 2-bromo-5-nitrothiazole as in example 5. Crystallization from ethanol and water gave 0.3 g of 3-hydroxy-5-[(5-nitrothiazol-2-yl)mercapto] -4-cyclohexyl- 1,2,4-triazole, a yellow solid, MP 237-239°C.

Example 7. 4-benzyl-3-hydroxy-5-[(5-nitrothien-2-yl)mercapto[-1,2,4-triazole

The title compound was prepared in a manner similar to that described in Example 5 starting with benzyl isothiocyanate. The intermediate 4-benzyl-3-thiosemicarbazide (1.81 g) was treated with ethyl chloroformate (1.09 g) as in Example 5. The reaction product, 4-benzyl-3-hydroxy-5-mercapto-1,2,4-triazole (1.04 g), was reacted with 1.05 g of 2-bromo-5-nitrothiazole as in Example 5. Crystallization from ethanol and water gave 0.3 g of 4benzyl-3-hydroxy-5-[(5-nitrothien-2-yl)mercapto]-l,2,4-triazole, a yellow solid, MP 221-224° C.

Example 8. 3-hydroxy-5-[(5-nitrothiazol-2-yl)mercapto]-4-[2-(trifluoromethyl) phenyl]-l,2,4-triazole

The title compound was prepared in a manner similar to that described in Example 5 starting with 2-(trifluoromethyl) phenyl isothiocyanate. The intermediate 4-[2-(trifluoromethyl)- phenyl]-3-thiosemicarbazide (2.04 g) was treated with ethyl chloroformate (1.09 g) as in example 5. The reaction product, 3-hydroxy-5-mercapto-4-[2-(trifluoromethyl)phenyl]-1,2,4-triazole (0.78 g) was reacted with 0.63 g of 2-bromo-5-nitrothiazole as in Example 5. Crystallization from ethanol and water gave 0.3 g

of 3hydroxy-5-[(5-nitrothiazol-2-yl)mercapto]-4-[2(trifluoro- methyl)phenyl]-1,2,4-triazole, a yellow solid, MP 183-185° C.

Example 9.

3-(l-ethyl-3-methylpyrazol-5-yl)-4-(3-methoxy-n-propyl)-5-[5-(nitrothiazol-2 yl)mercapto]-1,2,4-triazole

The title compound was synthesized in a manner similar to that described in Example 1. 3-Methoxy-n-propyl isothiocyanate was prepared from 3-methoxy-n-propylamine and thiophosgene at high temperature and then reacted with hydrazine in pyridine to give the intermediate 4-(3-methoxy-n-propyl)-3-thiosemicarbazide. 4-(3-Methoxy-n-propyl)-3-thiosemicarbazide (1.64 g) was reacted with 1-ethyl-3-methylpyrazole-5-carboxylic acid chloride (1.73 g, prepared from the acid and oxalyl chloride) to give 2 g of 1-(l-ethyl-3-methylpyrazole-5-carbonyl)-4-(3-methoxy-n-propyl)-3-thiosemicarbazide. Treatment of 1-(l-ethyl-3-methylpyrazole-5-carbonyl)-4-(3-methoxy-n-propyl)-3-thiosemicarbazide with potassium hydroxide in ethanol gave 3-(l-ethyl-3ethylpyrazol-5-yl)-5-mercapto-4-(3-methoxy-n-propyl)-1,2,4-triazole. Reaction of the sodium salt of 3-(l-ethyl-3-methylpyrazol-5-yl)-5-mercapto-4-(3-methoxy-n-propyl)-1,2,4-triazole(O.73 g) with 2-bromo-5-nitrothiazole (0.52 g) yielded crude 3-(l -ethyl-3-methylpyrazol-5-yl)-4-(3-methoxy-n-propyl)-5-[5-(nitrothiazol-2-yl)mercapto]-1,2,4-triazole as in Example 1. Crystallization from ethanol and water gave 0.3 g of 3-(l-ethyl-3-methylpyrazol-5-yl)-4-(3-methoxy-n-propyl)-5-[5-(nitrothiazol-2-yl)mercapto]-1,2,4-triazole, a light brown solid, MP 117-118° C.

Example 10. 3-(4-chlorophenyl)-5-[(5-nitrothiazol-2-yl)amino]-1,2,4-triazole

The title compound was prepared in a similar manner to that described in Example 1

by heating 3-amino-5-(4-chlorophenyl)-1,2,4-triazole with 2-bromo-5-nitrothiazole in refluxing tetrahydrofuran followed by silica gel column chromatograph using a mixture of dichloromethane and methanol as the eluent to yield 3-(4-chlorophenyl)-5-((5-nitrothiazo-2-yl)amino]-1,2,4-triazole.

Example 11. 4-Allyl-3-hydroxy-5-[5-nitrothien-2-yl)mercapto]-l,2,4-triazole

The title compound was prepared in a similar manner to that described in Example 5 starting with allyl isothiocyanate. 4-Allyl-3-hydroxy-5-mercapto-1,2,4-triazole was reacted with 2-bromo-5-nitrothiazole as in Example 5. Crystallization from ethanol and water gave 4-allyl-3-hydroxy-5-[(5nitrothien-2-yl)mercapto]-1,2,4-triazole as a yellow solid.

Example 12. 3-[(5-nitrothiazol-2-yl)mercapto]-4-(4 methoxyphenyl)-5-(thien-2-yl)-1,2,4- triazole

2-Bromo-5-nitrothiazole

To 72.5 g of 2 -amino-5-nitrothiazole in 300 mL of 48 % hydrobromic acid and 200 mL of water stirred and cooled to about –10° C was slowly added, in portions, 51.8 g of sodium nitrite dissolved in 80 mL of water from one addition funnel and 250 mL of n-amyl alcohol from a second addition funnel. The addition of both solutions required about 3 hours. The cooling bath was removed and the mixture allowed to warm to about 15° C overnight and then stirred at room temperature for 2 hours. The solid was collected by vacuum filtration and steam distilled to give 67 g of crude product. The crude product was recrystallized from hot ethanol to give 61 g (60 k yield) of the 2-bromo-5-nitrothiazole as a yellow solid.

N1-(4-Methoxyphenylaminothiocarbonyl)-N2-(thien-2carbonyl)hydrazine

Thien-2-carboxyhydrazide (2 g) and 2.3 g of 4methoxyphenylisothiocyanate in 25 mL

of tetrahydrofuran are stirred overnight at 25° C. The reaction is concentrated to give a precipitate which is collected by vacuum filtration and dried to give 3.5 g of the title compound as an off-white solid.

4-(4-Methoxyphenyl)-5-(thien-2-yl)-1,2,4-triazol-3-thione

Nl-(4-Methoxyphenylaminothiocarbonyl)-N2-(thien-2carbonyl)hydrazine (1 g) and 0.2 g of sodium ethoxide in 10 mL of ethanol are refluxed for 6 hours. The mixture is cooled and the precipitate collected by vacuum filtration and dried to give 0.6 g of the title compound as an off-white solid. 4-(4-Methoxyphenyl)-5-(thien-2-yl)-1,2,4-triazol-3-thione (0.5 g) and 0.4 g of 2-bromo-5-nitrothiazole in 50 mL of acetonitrile are refluxed for 6 hours. The solvent is concentrated to give crude product which is collected by vacuum filtration. The crude product is crystallized from ethanol and dried to give 0.6 g of the desired triazole compound as an off- white solid.

Example 13. 3-[(5-Nitrothiazole -2-yl)mercapto]-4-methyl-1,2,4-triazol

Methylaminothicarbonyl-hydrazine

By substituting 1 g of formic acid hydrazide for the 2 g of thien-2-carboxyhdrazide and 1 g of methylisothiocyanate for the 4-methoxyphenylisothiocyanate in the method of Example 12, 1 g of the title compound is obtained as an off-white solid.

Example 14. 3-[(5-nitrothiazol-2-yl)niercapto]-4-butyl-1,2,4-triazole

Butanoyl hydrazide

Butanoyl hydrazide is prepared according to the general method of A. I. Vogel, Practical Organic Chemistry, 3rd edition, 1956 (Longman Group, London) p 395. Ten grams of ethyl butanoate is refluxed in 10 mL of hydrazine hydrate for 15 minutes. Absolute ethanol is added, reflux is continued for 3 hours, and the ethanol distilled. The solution is

cooled and the crystalline hydrazide isolated by vacuum filtration and dried to give 8 g of butanoyl hydrazide. By substituting 1.7 g of butanoyl hydrazide for the formic acid hydrazide in Example 14, 0.6 g of the desired triazole compound is obtained as an off-white solid.

Example 15. 5-[(5-Nitrothiazol-2-yl)mercapto]-l-(4-methoxyphenyl)tetrazole

2-Bromo-5-nitrothiazole

To 72.5 g of 2-amino-5-nitrothiazole in 300 mL of 48% hydrobromic acid and 200 mL of water stirred and cooled to about –10° C was slowly added, in portions, 51.8 g of sodium nitrite dissolved in 80 mL of water from one addition funnel and 250 mL of n-amyl alcohol from a second addition funnel. The addition of both solutions required about 3 hours. The cooling bath was removed and the mixture allowed to warm to about 15° C overnight and then stirred at room temperature for 2 hours. The solid was collected by vacuum filtration and steam distilled to give 67 g of crude product. The crude product was recrystallized from hot ethanol to give 61 g (60 k yield) of the 2-bromo-5-nitrothiazole as a yellow solid.

1-(4-Methoxyphenyl)tetrazol-5-thione

4-Methoxyphenylisothiocyanate (2 g) and 1 g of sodium azide in 50 mL of ethanol is refluxed for 5 hours, cooled and concentrated (see, for instance, Canadian Journal of Chemistry 1959, p 101). The precipitate is collected by vacuum filtration and dried to give 1.5 g of 1-(4methoxyphenyl)tetrazol-5-thione as an off-white solid.

2-Bromo-5-nitrothiazole (1.1 g) and 1 g of 1-(4-methoxyphenyl)tetrazol-5-thione in 50 mL of acetonitrile are refluxed for 5 hours and the acetonitrile evaporated. The residue is crystallized from ethanol to give 1.2 g of the desired tetrazole compound as an off-white solid.

Example 16. 2-[(5-Nitrothiazol-2-yl)mercapto]-5-methyl-1,3,4-thiadiazole

2-Bromo-5-nitrothiazole

To 72.5 g of 2-amino-5-nitrothiazole in 300 mL of 48 % hydrobromic acid and 200 mL of water stirred and cooled to about –10° C was slowly added, in portions, 51.8 g of sodium

nitrite dissolved in 80 mL of water from one addition funnel and 250 mL of n-amyl alcohol from a second addition funnel. The addition of both solutions required about 3 hours. The cooling bath was removed and the mixture allowed to warm to about 15° C overnight and then stirred at room temperature for 2 hours. The solid was collected by vacuum filtration and steam distilled to give 67 g of crude product. The crude product was recrystallized from hot ethanol to give 61 g (60 % yield) of 2-bromo-5-nitrothiazole as a yellow solid.

2-Mercapto-5-methyl-1,3,4-thiadiazole

2-Mercapto-5-methyl-1,3,4-thiadiazole is prepared according to the general method described by S. G. Boots and C. C. Cheng, 1967, J. Hetercyclic Chemistry, 4: 272-283. Acetic acid hydrazide (7.4 g) 160 mL of methanol, 5.0 g of 85 % potassium hydroxide pellets, and 10 mL of carbon disulfide is stirred at room temperature for 4 hours. Ether (400 mL) is added and and the mixture is cooled in an ice bath to give 10 g of solid potassium acetyldithiocabazate, which is collected by vacuum filtration, dried, and used immediately. The crude potassium acetyldithiocarbazate in a mixture of 300 mL of dichloromethane and 54 mL of boron trifluoride etherate is stirred under nitrogen for 18 hours. The orange solution is poured onto ice and extracted with ether. The ether extract is washed with 10 % potassium hydroxide solution and the aqueous phase acidified to pH 2 with cold 10 % hydrochloric acid. The precipitate is collected by vacuum filtration and dried to give 2.5 g of the title compound as a beige solid.

2-Bromo-5-nitrothiazole (2.09 g) and 1.32 g of 2-mercapto-5-methyl-1,3,4-thiadiazole in 10 mL of tetrahydrofuran are stirred at room temperature overnight, triethylamine is added to neutrality, and stirring is continued. The precipitated solid is dissolved in dichloromethane, washed with water, and the organic layer evaporated to give crude product. The crude product is crystallized from ethyl acetate/hexane to give 1 g of desired thiazole-thiadiazole compound as an off-white solid.

Alternatively, 2-[(5-nitrothiazol-2-yl)mercapto]-5-methy-1,3,4-thiadiazole is prepared by the general method of J. Bourdais, et al., 1981, Eur. J. Chem. Chim. Ther., 16: 233-240. 2Bromo-5-nitrothiazole (2.09 g) and 1.32 g of 2-mercapto-5methyl-1,3,4-thiadiazole in 10 mL of ethanol and 10 mL of 1 N potassium hydroxide are stirred at room temperature overnight. The precipitated solid is collected by vacuum filtration, washed with water and crystallized from ethyl acetate/hexane to give 1 g of the title compound as an off-white solid.

Alternatively, 2-[(5-nitrothiazol-2-yl)mercapto]-5-methyl-1,3,4-thiadiazole is prepared by forming and isolating the sodium salt of 2-mercapto-5-methyl-1,3,4-thiadiazole, reacting it with 2-bromo-5-nitrothiazole in a suitable inert solvent at room temperature and isolating the product as described above. For an example of this method see M. F. Abdel-Lateef and Z. Eckstein, 1972, Rocz. Chem., 46: 1647- 1658.

Example 17. 2-[(5-Nitrothiazol-2-yl)mercapto-5-propyl-1,3,4-thiadiazole

Butanoyl hydrazide is prepared according to the general method of A. I. Vogel, Practical Organic Chemistry, 3rd edition, 1956 (Longman Group, London) p 395. Ten grams of ethyl butanoate is refluxed in 10 mL of hydrazine hydrate for 15 minutes. Absolute ethanol is added, reflux is continued for 3 hours, and the ethanol distilled. The solution is cooled and the crystalline hydrazide isolated by vacuum filtration and dried to give 8 g of

propanoyl hydrazide.

2-Mercapto-5-propyl-1,3,4-thiadiazole is prepared as for 2--mercapto-5-methyl-1,3,4-thiadiazole by substituting propanoyl hydrazide for acetic hydrazide in Example 16.

By substituting 2-mercapto-5-propyl-1,3,4-thiadiazole for 2 -mercapto-5-methyl-1,3,4-thiadiazole in Example 17, 2-[(5-nitrothiazol-2-yl)mercapto]-5-propyl-1,3,4-thiadiazole is isolated as an off-white solid.

BRIEF DESCRIPTION OF THE TABLES.

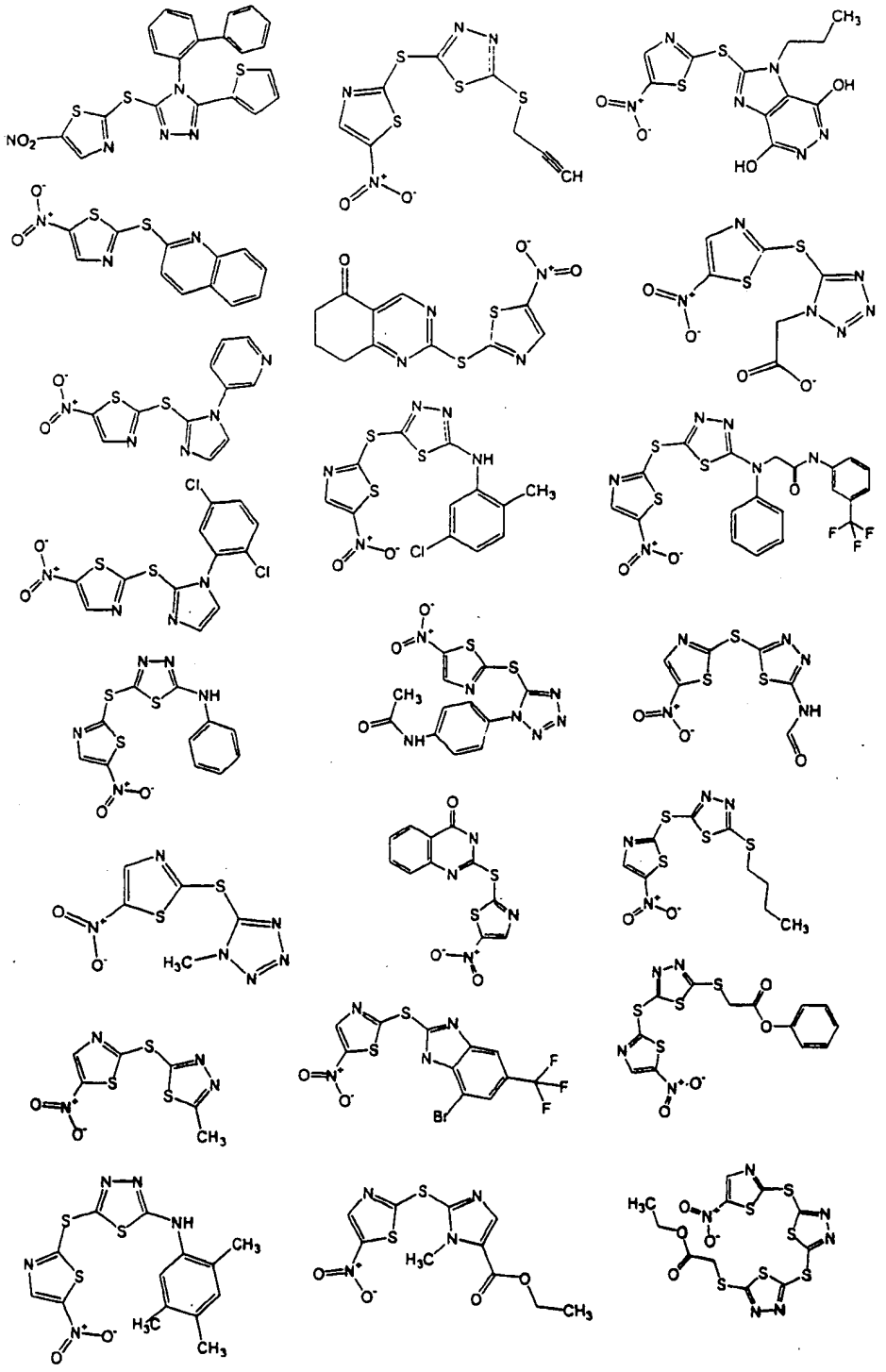
Table 1 shows preferred chemical structures which are within the scope of this invention. The compounds shown are in no way to be construed as limiting the scope of this invention.

BIOLOGICAL EVALUATION

It will be appreciated that, in any given series of compounds, a spectrum of biological activity will be observed. In a preferred embodiment, the present invention relates to novel heteroaryl compounds demonstrating the ability to modulate protein tyrosine enzymes related to cellular signal transduction, most preferrably, protein tyrosine phosphatase. The assays described below are employed to select those compounds demonstrating the optimal degree of the desired activity .

As used herein, the phrase "optimal degree of desired activity" refers to the highest therapeutic index, defined above, against a protein tyrosine enzyme which mediates cellular signal transduction and which is related to a particular disorder so as to provide a patient, preferably a human, suffering from such disorder with a therapeutically effective amount of a compound of this invention at the lowest possible dosage.

TABLE 1



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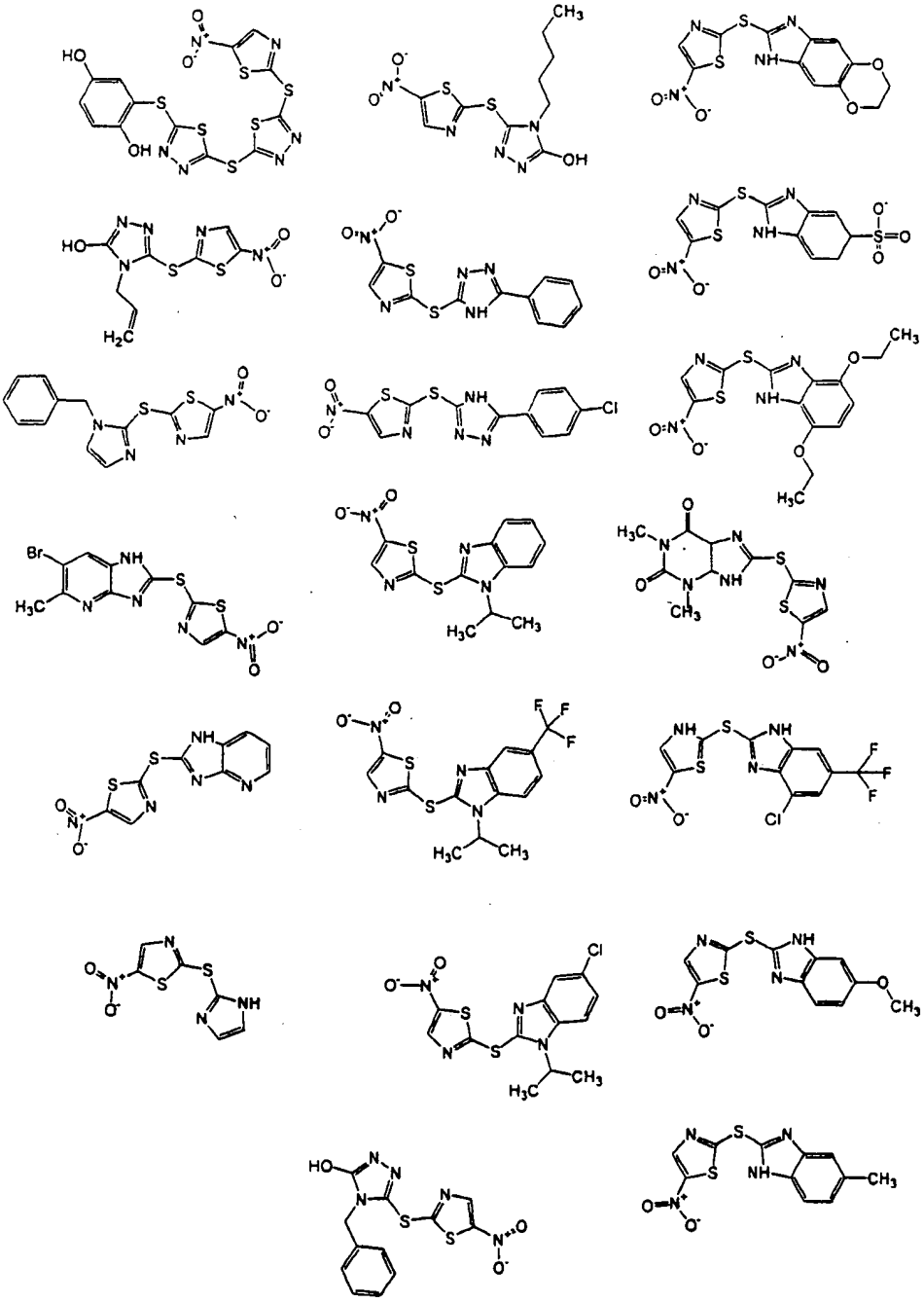

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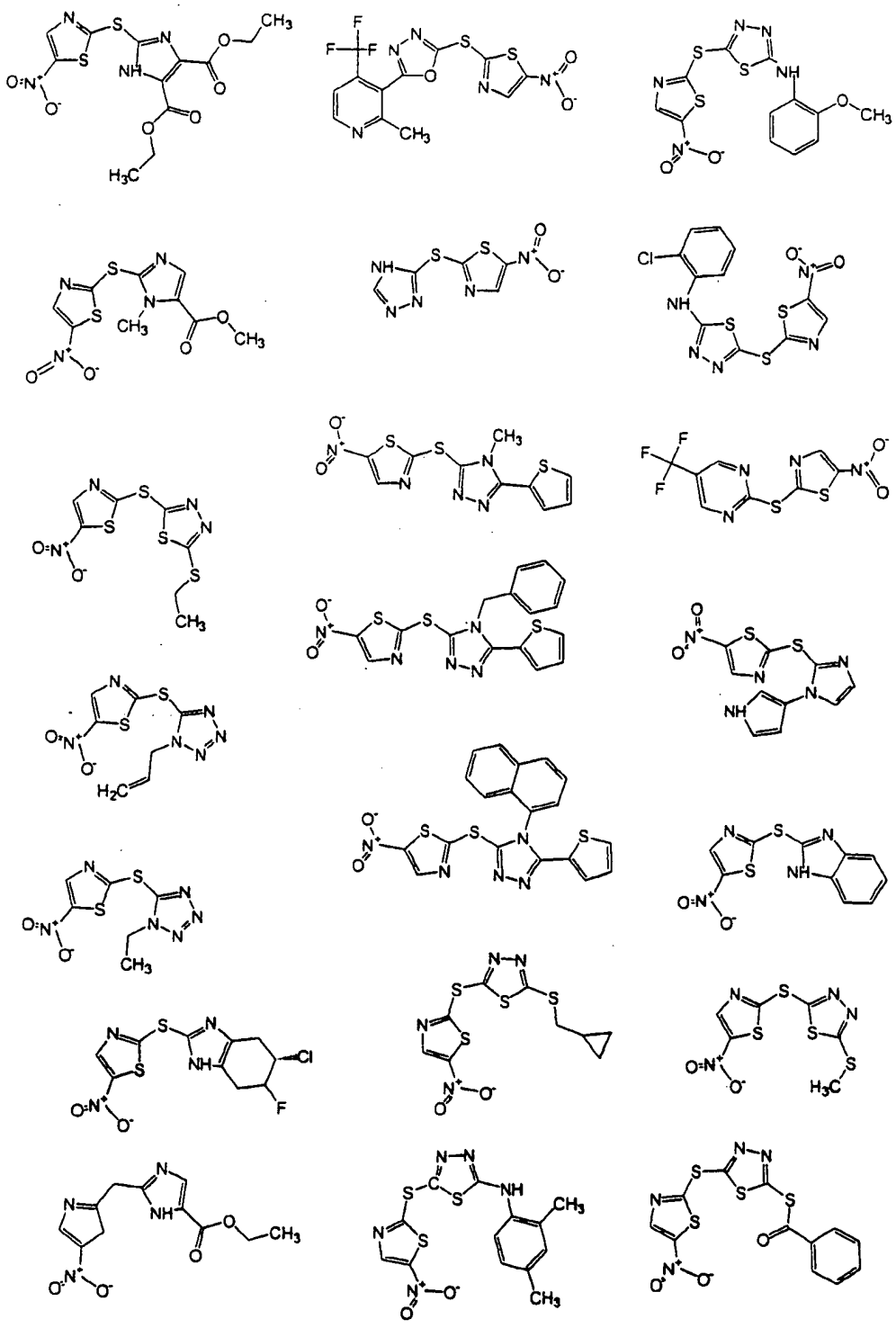

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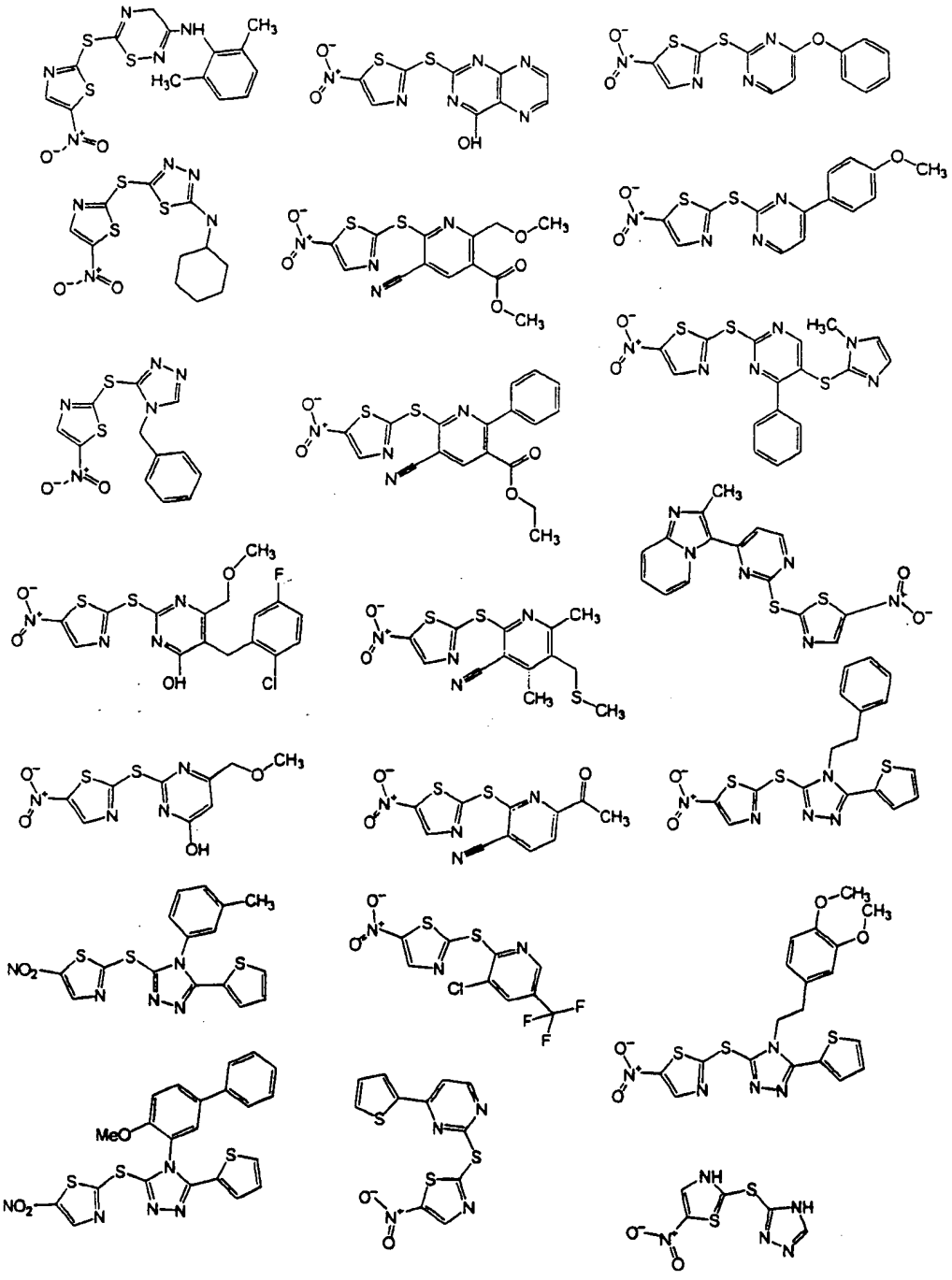

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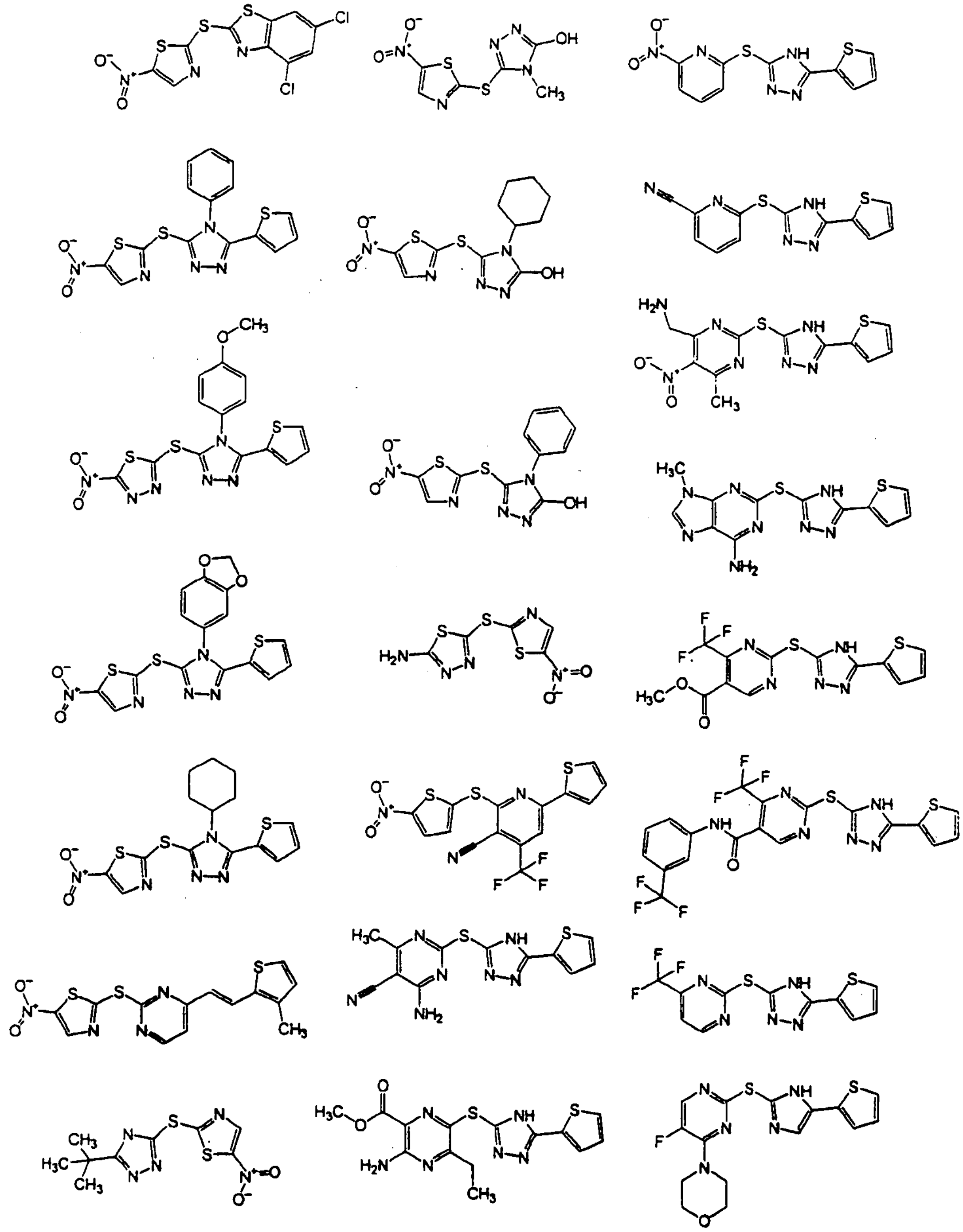

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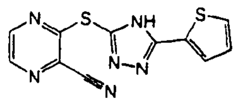

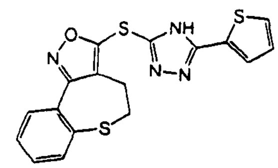

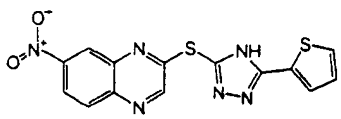

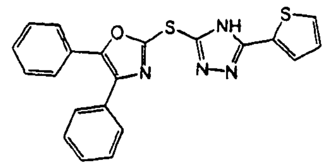

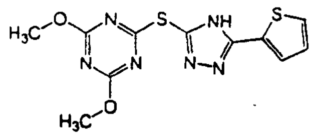

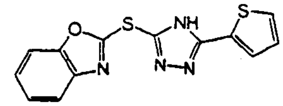

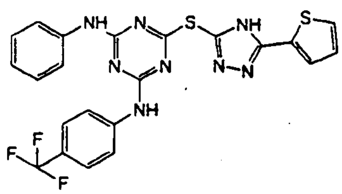

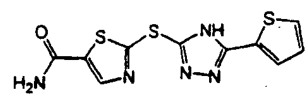

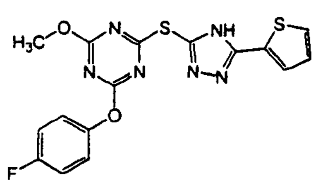

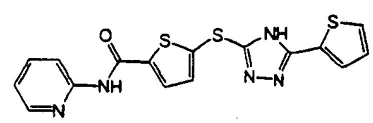

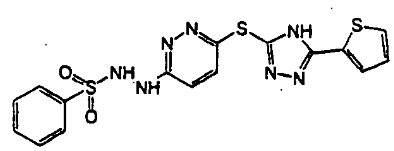

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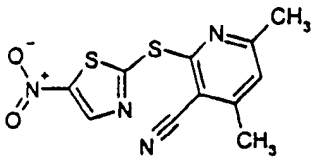

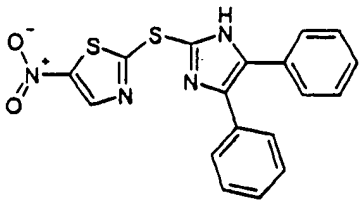

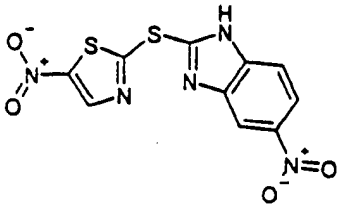

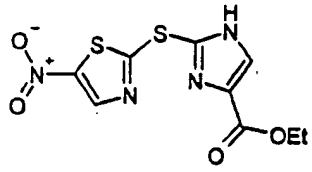

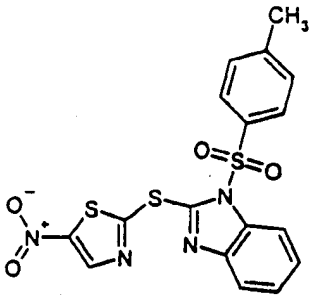

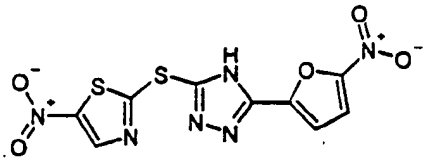

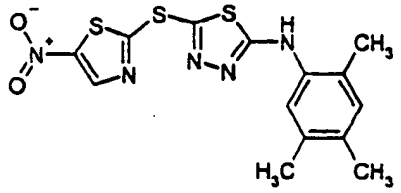

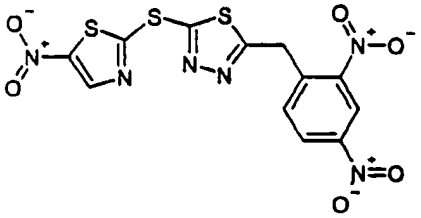

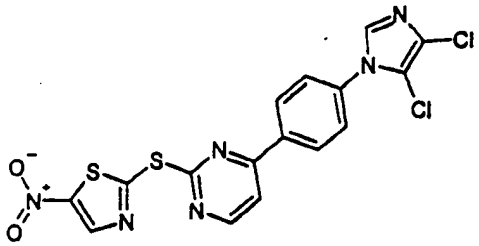

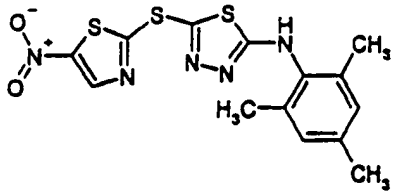

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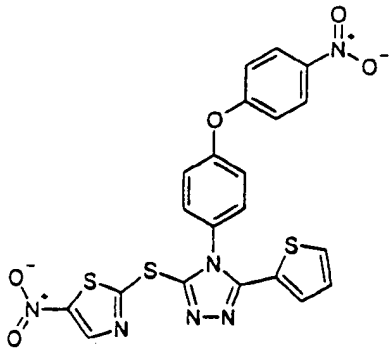

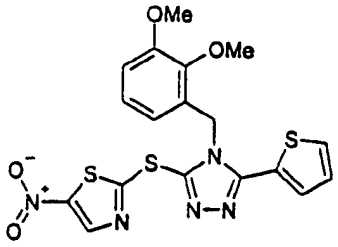

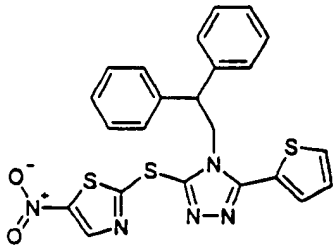

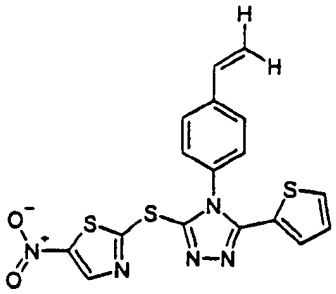

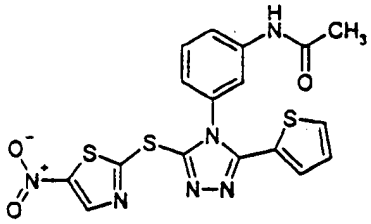

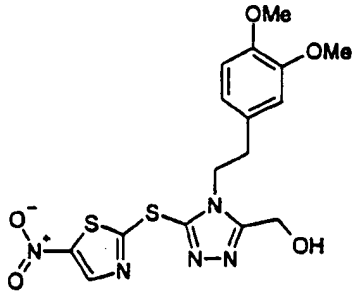

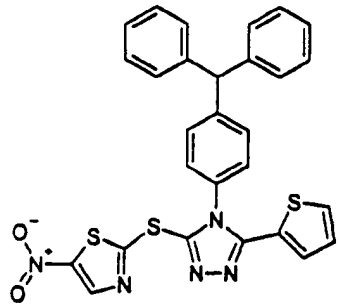

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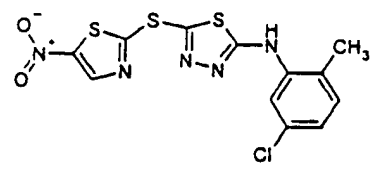

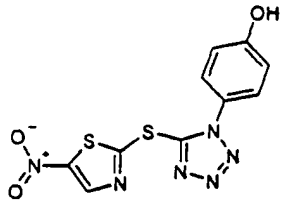

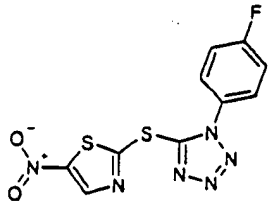

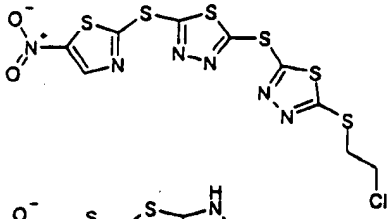

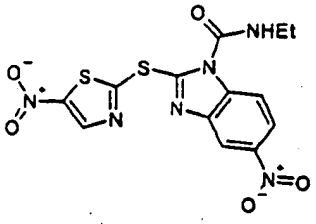

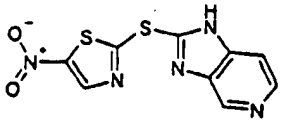

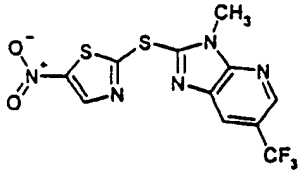

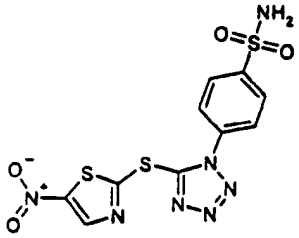

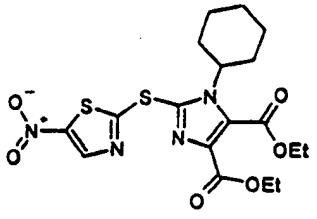

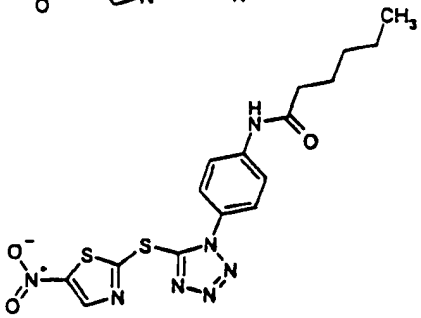

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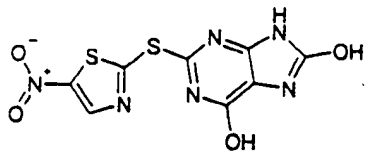

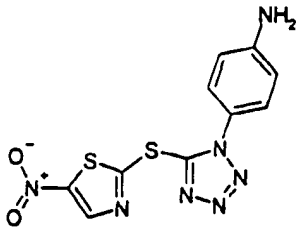

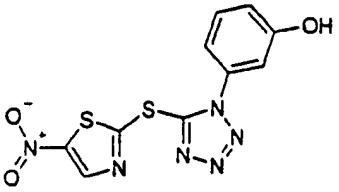

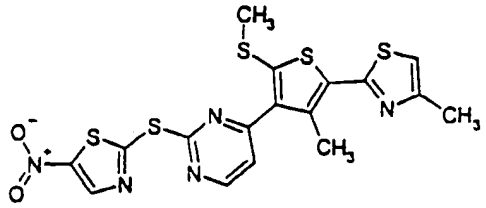

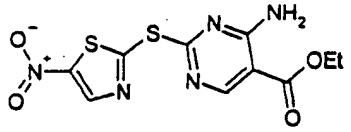

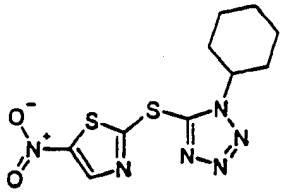

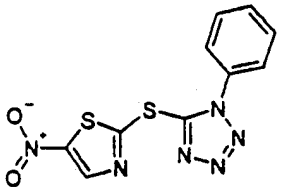

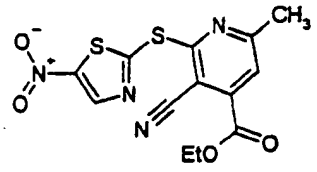

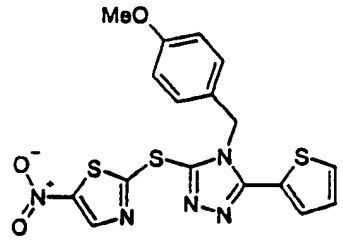

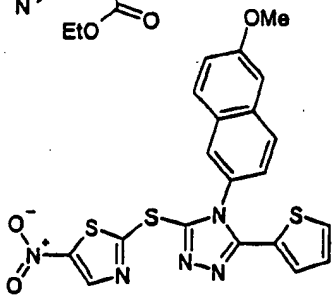

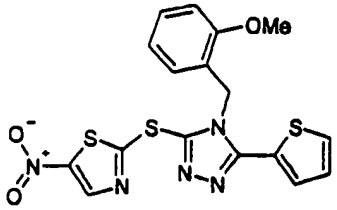

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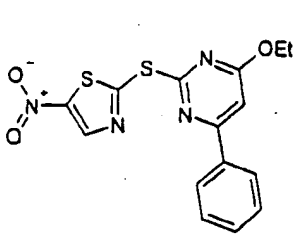
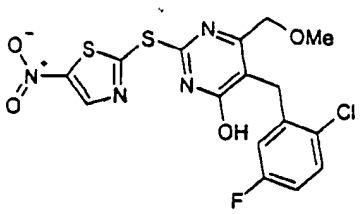
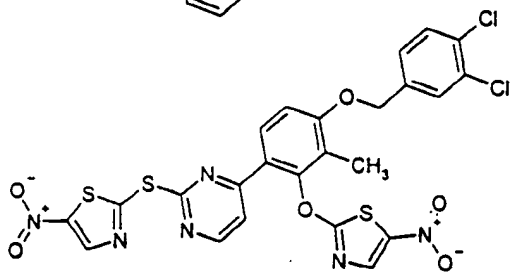
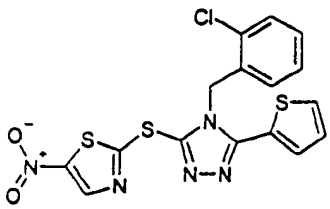
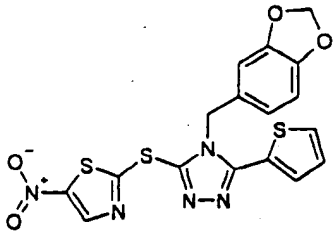
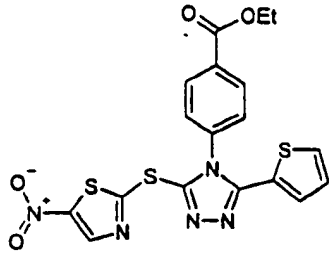
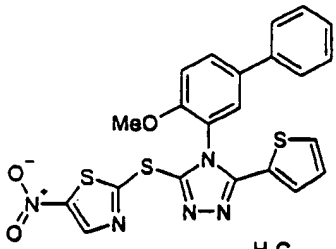
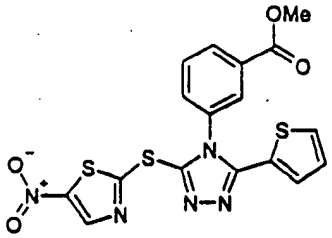
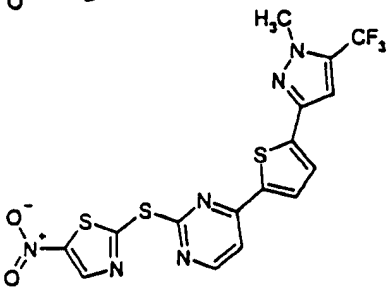
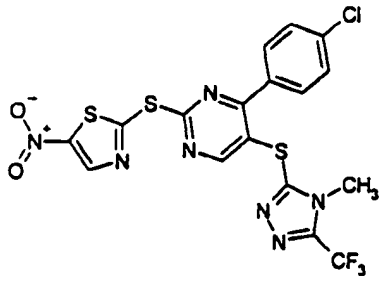

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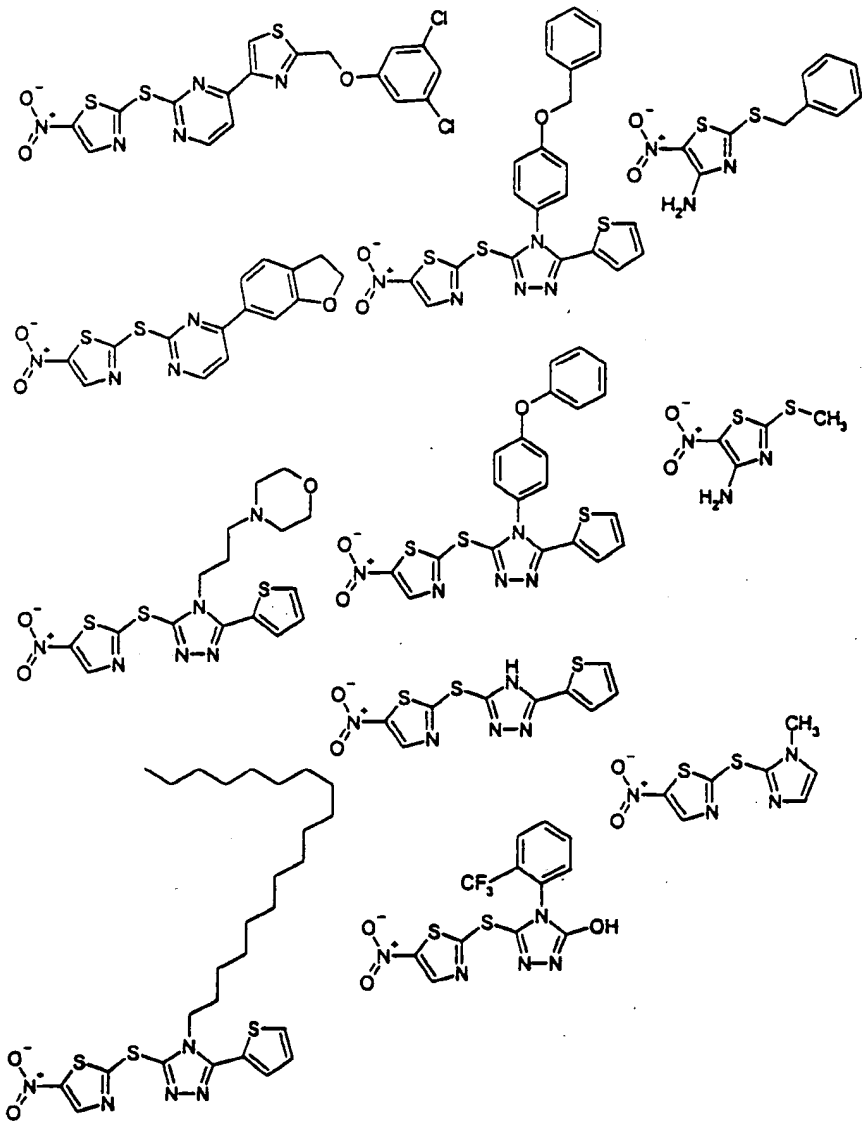

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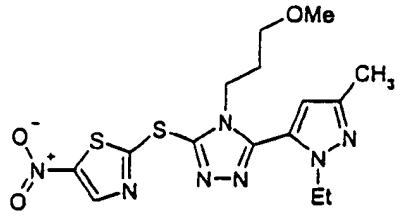
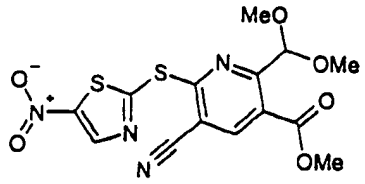
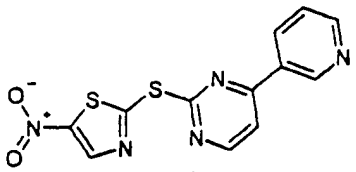
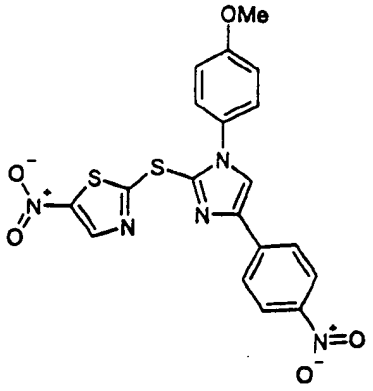
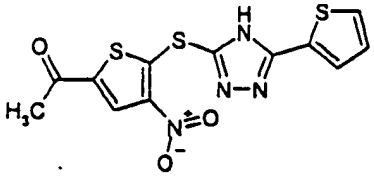
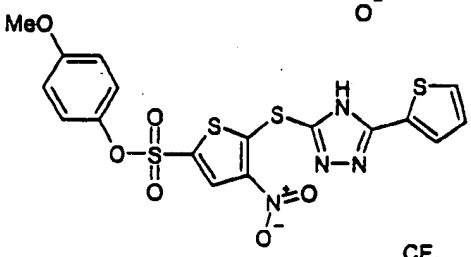
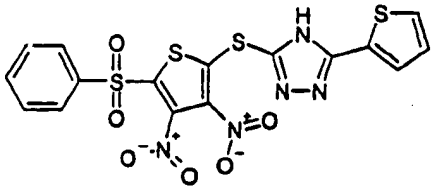
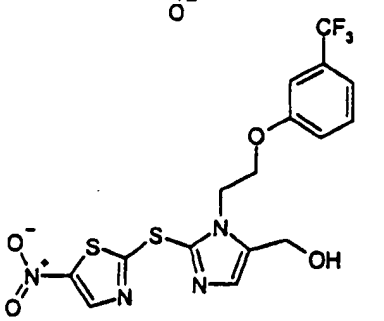
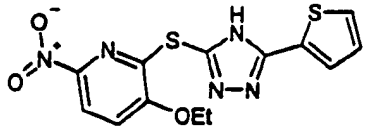

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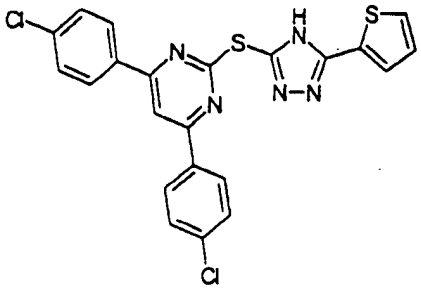
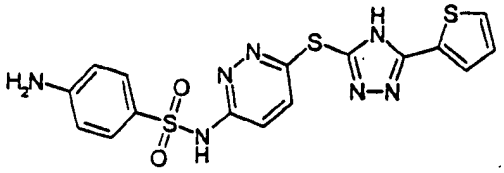
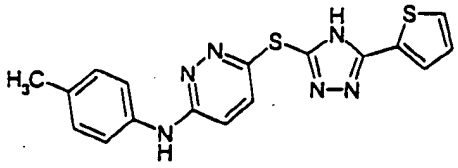
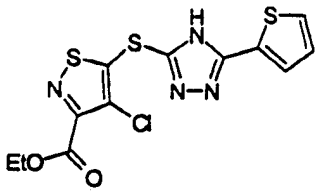
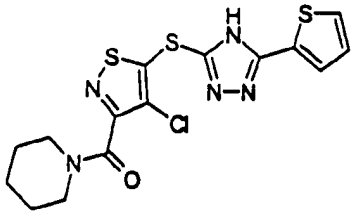
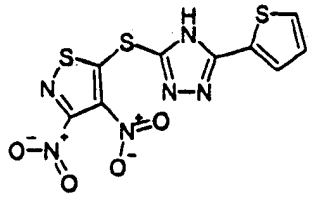












Assays For Determining Inhibitory Activity

Various procedures known in the art may be used for identifying, evaluating or assaying the inhibition of activity of protein tyrosine enzymes, in particular protein tyrosine phosphatases, by the compounds of the invention. For example but without limitation, with regard to phosphatases such assays involve exposing target cells in culture to the compounds and a) biochemically analyzing cell lysates to assess the level and/or identity of tyrosine phosphorylated proteins; or (b) scoring phenotypic or functional changes in treated cells as compared to control cells that were not exposed to the test substance.

Where mimics of the natural ligand for a signal transducing receptor are to be identified or evaluated, the cells are exposed to the compound of the invention and compared to positive controls which are exposed only to the natural ligand, and to negative controls which were not exposed to either the compound or the natural ligand. For receptors that are known to be phosphorylated at a basal level in the absence of the natural ligand, such as the insulin receptor, the assay may be carried out in the absence of the ligand. Where inhibitors or enhancers of ligand induced signal transduction are to be identified or evaluated, the cells are exposed to the compound of the invention in the presence of the natural ligand and compared to controls which are not exposed to the compound of the invention.

The assays described below may be used as a primary screen to evaluate the ability of the compounds of this invention to inhibit phosphatase activity of the compounds of the invention. The assays may also be used to assess the relative potency of a compound by testing a range of concentrations, in a range from 100 μM to 1 pM, for example, and computing the concentration at which the amount of phosphorylation or signal transduction is reduced or increased by 50% (IC50) compared to controls.

Biochemical Assays

Target cells having a substrate molecule that is phosphorylated or dephosphorylated

on a tyrosine residue during signal transduction are exposed to the compounds of the invention and radiolabelled phosphate, and thereafter, lysed to release cellular contents, including the substrate of interest. The substrate may be analyzed by separating the protein components of the cell lysate using a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique, in either one or two dimensions, and detecting the presence of phosphorylated proteins by exposing to X-ray film. In a similar technique, but without radioactive labeling, the protein components separated by SDS-PAGE are transferred to a nitrocellulose membrane, the presence of pTyr is detected using an antiphosphotyrosine (anti-pTyr) antibody. Alternatively, it is preferred that the substrate of interest be first isolated by incubating the cell lysate with a substrate-specific anchoring antibody bound to a solid support, and thereafter, washing away non-bound cellular components, and assessing the presence or absence of pTyr on the solid support by an anti-pTyr antibody. This preferred method can readily be performed in a microtiter plate format by an automated robotic system, allowing for testing of large numbers of samples within a reasonably short time frame.

The anti-pTyr antibody can be detected by labeling it with a radioactive substance which facilitates its detection by autoradiography. Alternatively, the anti-pTyr antibody can be conjugated with an enzyme, such as horseradish peroxidase, and detected by subsequent addition of an appropriate substrate for the enzyme, the choice of which would be clear to one skilled in the art. A further alternative involves detecting the anti-pTyr antibody by reacting with a second antibody which recognizes the anti-pTyr antibody, this second antibody being labelled with either a radioactive substance or an enzyme as previously described. Any other methods for the detection of an antibody known in the art may be used.

The above methods may also be used in a cell-free system wherein cell lysate containing the signal-transducing substrate molecule and phosphatase is mixed with a compound of the invention and a kinase. The substrate is phosphorylated by initiating the

kinase reaction by the addition of adenosine triphosphate (ATP). To assess the activity of the compound, the reaction mixture may be analyzed by the SDS-PAGE technique or it may be added to a substrate-specific anchoring antibody bound to a solid support, and a detection procedure as described above is performed on the separated or captured substrate to assess the presence or absence of pTyr. The results are compared to those obtained with reaction mixtures to which the compound is not added. The cell-free system does not require the natural ligand or knowledge of its identity. For example, Posner et al. (U.S. Patent No. 5,155,031) describes the use of insulin receptor as a substrate and rat adipocytes as target cells to demonstrate the ability of pervanadate to inhibit PTP activity. Burke et al., Biochem. Biophys. Res. Comm., 1994, 204:129-134) describes the use of autophosphorylated insulin receptor and recombinant PTP1B in assessing the inhibitory activity of a phosphotyrosyl mimetic.

In addition to measuring phosphorylation or dephosphorylation of substrate proteins, activation or modulation of second messenger production, changes in cellular ion levels, association, dissociation or translocation of signaling molecules, gene induction or transcription or translation of specific genes may also be monitored. These biochemical assays may be performed using conventional techniques developed for these purposes.

Biological Assays

The ability of the compounds of this invention to modulate the activity of PTPs, which control signal transduction, may also be measured by scoring for morphological or functional changes associated with ligand binding. Any qualitative or quantitative techniques known in the art may be applied for observing and measuring cellular processes which come under the control of phosphatases in a signaling pathway. Such cellular processes may include, but are not limited to, anabolic and catabolic processes, cell proliferation, cell differentiation, cell adhesion, cell migration and cell death.

The techniques that have been used for investigating the various biological effects of vanadate as a phosphatase inhibitor may be adapted for use with the compounds of the invention. For example, vanadate has been shown to activate an insulin-sensitive facilitated transport system for glucose and glucose analogs in rat adipocytes (Dubyak, et al., 1980, J. Biol. Chem., 256:5306-5312). The activity of the compounds of the invention may be assessed by measuring the increase in the rate of transport of glucose analog such as 2-deoxy-^{3}Hglucose in rat adipocytes that have been exposed to the compounds. Vanadate also mimics the effect of insulin on glucose oxidation in rat adipocytes (Shechter, et al., 1980, Nature, 284:556-558). The compounds of this invention may be tested for stimulation of glucose oxidation by measuring the conversion of ^{14}C-glucose to $^{14}CO_2$. Moreover, the effect of sodium orthovanadate on erythropoietin-mediated cell proliferation has been measured by cell cycle analysis based on DNA content as estimated by incorporation of tritiated thymidine during DNA synthesis (Spivak, et al., 1992, Exp. Hematol., 20:500-504). Likewise, the activity of the compounds of this invention toward phosphatases that play a role in cell proliferation may be assessed by cell cycle analysis.

The activity of the compounds of this invention can also be assessed in animals using experimental models of disorders caused by or related to dysfunctional signal transduction. For example, the activity of a compound of this invention may be tested for its effect on insulin receptor signal transduction in non-obese diabetic mice (Lund et al., 1990, Nature, 345:727-729), B B Wistar rats and streptozotocin-induced diabetic rats (Solomon et al., 1989, Am. J. Med. Sci., 297:372-376). The activity of the compounds may also be assessed in animal carcinogenesis experiments since phosphatases can play an important role in dysfunctional signal transduction leading to cellular transformation. For example, okadaic acid, a phosphatase inhibitor, has been shown to promote tumor formation on mouse skin (Suganuma et al., 1988, Proc. Natl. Acad. Sci., 85:1768-1771).

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of the compounds of the invention should lie within a range of circulating concentrations with little or no toxicity. The dosage may vary within this range depending on the dosage form employed and the route of administration.

The above-described assays are exemplary and not intended to limit the scope of the invention in any manner. Other assays known to those skilled in the art may be employed to ascertain the ability of the compounds of this Those of skill in the art would appreciate that modifications can be made to the assays to develop equivalent assays that obtain the same result.

Phosphatase Inhibitors

The present invention encompasses compounds capable of regulating and/or modulating signal transduction by, including but not limited to, inhibiting the activity of protein tyrosine phosphatases. More specifically, the present invention encompasses compounds capable of inhibiting protein tyrosine phosphatase activity. These compounds will be referred to herein generically as "phosphatase inhibitors", even though these compounds either upregulate or downregulate cellular processes that are controlled by signal transduction.

Phosphotyrosine Enzyme Linked Immunosorbent Assay

This assay may be used to test the ability of the compounds of the invention to inhibit dephosphorylation of phosphotyrosine (ptyr) residues on insulin receptor (IR). Those skilled in the art will recognize that other substrate molecules, such as platelet derived growth factor receptor, may be used in the assay by using a different target cell and anchoring antibody. By using different substrate molecules in the assay, the activities of the compounds of this invention toward different protein tyrosine enzymes may be assessed. In the case of IR, an

endogenous kinase activity is active at low level even in the absence of insulin binding. Thus, no insulin is needed to stimulate phosphorylation of IR. That is, after exposure to a compound, cell lysates can be prepared and added to microtiter plates coated with anti-insulin receptor antibody. The level of phosphorylation of the captured insulin receptor is detected using an anti-pTyr antibody and an enzyme-linked secondary antibody.

Materials And Methods

1. The cell line used for the IR assay is NIH3T3 cells (ATCC# CRL 1658) engineered to over-express the human IR (H25 cells). Growth media for these cells is DMEM (Gibco) containing 10% fetal bovine serum, 1% L-glutamine, and 20 mM Hepes.

2. The anchoring antibody used was BBE which recognizes the extracellular domain of human IR (Enzymology Laboratories, Sugen Inc.).

3. PBS (Gibco): KH_2PO_4 (0.20 g/l), K_2HP0_4 (2.16 g/l), KCl (0.20 g/l), NaCl (8.0 g/l), pH 7.2.

4. Rabbit polyclonal antiphosphotyrosine antibody (anti-pTyr, Enzymology Laboratories, Sugen, Inc.).

5. Goat anti-rabbit IgG POD conjugate (Tago, Burlingame, CA, Cat.No. 6430) is used as the secondary antibody.

6. TBST buffer: 50 mM Tris-HCl, 150 mM NaCl, 0.1% Triton X-100, adjusted to pH 7.2 with 10N HCl.

7. Blocking buffer: PBS plus 5% milk (Carnation instant non-fat dry milk).

8. 5X HNTG buffer: 100 mM HEPES, 750 mM NaCl, 50% glycerol, 0.5% Triton X-100, pH 7.5.

9. ABTS solution: 100 mM citric acid, 250 mM Na_2HP0_4, 0.5 mg/ml ABTS (2,2'-azinobis(3-ethylbenzthiazlinesulfonic acid), adjusted to pH 4.0 with 1N HCl.

10. Cell lysis buffer: HNTG containing lmM Na_3VO_4, (0.5M solution kept as a

100X stock at –80°C in aliquots), 5mM NaP_2O_7 and 5mM EDTA prepared fresh and keep on ice until ready for use.

11. Hydrogen peroxide: 30% solution.

Preparation Of Assay Plates

Microtiter plates (96-well, Easy Wash ELISA plate, Corning 25805-96) are coated with the anchoring antibody at 0.5 µg per well, in 100 µl PBS for at least two hours at room temperature or overnight at 4° C. Before use, the coating buffer is replaced with 100 µl blocking buffer, and the precoated assay plate was shaken at room temperature for 30 minutes. The wells are then washed 3 times with water and once with TBST buffer before adding lysate.

Seeding Cells

Cells are grown in a 15cm culture dish (Corning 25020-100) in DMEM media containing 10% fetal bovine serum (FES) until 80-90% confluent. The cells are harvested with trypsin-EDTA (0.25%, 0.5ml, Gibco), resuspended in fresh medium containing 10% FBS, 1% L-glutamine and Hepes, and transferred to round bottom 96-well tissue culture plates (Corning 25806-96) at 25,000 cells/well, 100 µl/well. The cells are incubated at 37° C at 5% $C0_2$ for 24 hours. The media is then changed by inverting the plate, and adding DMEM medium containing 0.5% FBS and Hepes. The cells are further incubated overnight at 37°C, 5% $C0_2$.

Assay Procedure

The assay is set up in the 96-well tissue culture plate. Before adding the compounds to the cells, media in the wells is replaced by serum free DMEM medium, 90 µl per well. Positive control wells receive 80 µl DMEM. Negative controls received 90 µl DMEM. The test compound is diluted 1:10 with DMEM and 10 µl/well of the diluted test substances are transferred to the cells in the wells to achieve a final dilution of 1:100. Positive and negative

control wells both receive 10µl/well of dimethyl sulfoxide (DMSO) to achieve a final concentration of 1%. Positive control wells additionally received 10 µl/welll of O.lM Na_3VO_4 so that that the final concentration is l0 mM. The tissue culture plate is shaken for 1 minute before incubation at 37°C, 5% C02. After 90 minutes of incubation, the media is removed by inversion of the plate, and l00µl/well of lysis buffer is added. The tissue culture plate is shaken for 5 minutes and then placed on ice for 10 minutes. The cells are homogenized by repeated aspirating and dispensing, and the lysate is then transferred to the corresponding wells of a precoated assay plate.

The substrate in the cell lysates is allowed to bind to the anchoring antibody for 1 hour with shaking at room temperature. The lysate is then removed, and the assay plate is washed. All ELISA plate washings are done by rinsing in water 3 times followed by one rinse with TBST. The plate is dried by tapping it on paper towels. Phosphotyrosine is detected by adding 100 µl/well anti-pTyr antiserum diluted 1:3000 with TBST to the wells and incubating for 30 minutes shaking at room temperature. The unbound excess anti-pTyr antiserum is then removed, and the assay plate is washed as described above. A secondary antibody diluted 1:3000 with TBST, is added to the wells and incubated for 30 minutes with shaking at room temperature. The secondary antibody is then removed, the plate washed, and fresh ABTS/H_20_2 (1.2 µl 30% H_20_2 to l0 ml 0.5 mg/ml 2,2'-azinobis(3-ethylbenzethiazline)sulfonic acid in l00 mM citric acid, 250 mM Na_2HPO_4, pH 4.0) is added to start color development. The reaction is stopped after 10 minutes by adding 100 µl/well of 0.2M HC1, and incubating with shaking for 1 minute. Absorbance values at 410 nm were measured using an ELISA plate reader (Dynatec MR5000).

Glucose Transport Assay

This assay is used to assess the ability of the compounds of the invention to inhibit phosphatase activity that is involved in the signaling pathway that regulates the

insulin-induced transport of glucose into adipocytes. It has been shown that incubation of isolated adipocytes with vanadate resulted in a dose-dependent increase in the rate of glucose uptake.

Materials And Methods

The cell line used for the glucose transport assay is 3T3-L1, a preadipocyte cell line (American Type Culture Collection CCL92.1) which overexpress the insulin receptor. The 3T3-L1 cells are first differentiated by treating the cells under confluent growth conditions in DMEM containing 10% fetal bovine serum (FBS) with 0.5 mM 3-isobutyl-1-methylxanthine, 5 µl/ml porcine insulin and 250 mM dexamethasone for 2 days. The cells are then grown in DMEM containing 10% FBS and 5 µl/ml porcine insulin for two days, after which the cells are cultured in DMEM containing only 10% FBS.

Cells for use in the assay are first grown overnight in DMEM media and 1% FBS at 37°C at 5% CO_2. Two hours before use, the overnight media is replaced with serum free DMEM containing 5mM glucose. After washing the cells twice with phosphate buffered saline (PBS), serial dilutions of the test compound 1:100 into DMEM are added to the wells for a final concentration range of 0.1 µM to 500 µM. Negative control wells received DMEM only. The cells are incubated with the test compound for 1-4 hours at 37°C at 5% $C0_2$. Fifteen minutes before the end of the selected time, 2-deoxy-^{3}H-glucose is added to a final concentration of 50 µM and 0.5 µCi/ml. At the end, the compound is removed and the wells are washed twice with PBS containing 10 mM glucose. The cells are lysed with 5Oµl 0.5N NaOH and the cell lysates are transferred to a scintillation vial and mixed with 5.2 µl of glacial acetic acid. The wells are each washed with 200 µl PBS which is then transferred to the corresponding scintillation vial. 3H radioactivity is then counted with a Beckman counter.

Primary Adipocyte Glucose Uptake Assay

This assay is used to assess the effect of the compounds of the invention on insulin-mediated signal transduction of primary adipocytes as determined by glucose uptake by the cells.

MATERIALS AND METHODS

Reagents

The following buffers and solutions are used in the primary adipocyte glucose uptake assay:

Mixed Salts

76.74g NaCl

3.51g KCl

3.06g $MgSO_4 \cdot 7H_2O$

3.63g $CaCl_2 \cdot 2H_2O$ (2.74g $CaCl_2$)

The volume was brought up to 1 liter with distilled H_2O.

HEPES Buffer

23.8g HEPES

3.42g $NaH_2PO_4 \cdot H_2O$ (3.87g $NaH_2PO_4 \cdot 2H_2O$)

were dissolved in approximately 600ml of H_2O. The pH of the solution was adjusted to 7.6 and the volume was brought up to 1 liter with distilled H_2O.

Albumin Collagenase Buffer

44.8ml distilled H_2O

10.0 ml HEPES Buffer

10.0 ml Mixed Salts

35.0 ml 10% BSA

0.2ml Glucose (300mM)

100mg Collagenase

Transport buffer

48ml distilled H_20

8ml Mixed salts

8ml HEPES Buffer

16ml 10% Bovine Serum Albumin

Excision of epididymal fat pads

Primary adipocytes used in the assay are obtained from euthanized male rats (Sprague-Dawley or other appropriate strains) with a body weight of 200-250 grams. Old and heavier rats are not used as these rats may be resistant to insulin and do not provide a good response. Each animal is expected to yield 1-1.5 g of fat. Approximately 2.5 g of fat is required to run 40 reactions, with 20 samples in the glucose uptake assay and a matching set of 20 LDH samples.

Using sterile techniques, a midline abdominal incision is made through the skin followed by a 4-6 cm incision through the peritoneum. The fat body adjoining the testes is identified by tracing the vas deferens to the testes. The fat pads are carefully cut away from the epididymis and testes, and the innervating blood vessels. The excised fat pads are weighed, finely chopped, and digested with 5 ml of collagenase buffer at 37°C for 1 hour. The digested material was then strained through a 250-micron nylon mesh sieve. The cells float to the top, and are collected and washed three times with transport buffer. The cell concentration is determined by one of the following methods:

(1) Cells as percentage of solution: The cells in suspension are centrifuged at 500x g for 5-10 minutes in a hematocrit tube. The total length of the column of liquid and the length of column of "white" cellular material at the bottom of the tube is measured in millimeters.

The cell concentration is estimated as a percentage of the length of cell column to the total length. For the glucose uptake assay, approximately 2-3% of the cells in the final reaction volume are used. For a 500 µl reaction volume, the fat cell stock solution is diluted to 25% cells with transport buffer, and 50 µl aliquots are added to each sample.

(2) Cell Number: Cells are first fixed with osmium tetroxide in collidine buffer so that the adipocytes sink in suspension. The fixed cells are centrifuged to remove the osmium tetroxide, and then counted with a Coulter counter. Once the cell number is determined, the cell concentration is adjusted and 50 µl aliquots of the cells are added to each sample.

Assay

Adipocytes collected from rats are exposed to a test compound in the absence or presence of saturation levels of insulin. ^{14}C-labelled glucose, which would normally be taken up by the cells via an insulin-induced mechanism, are added to the cells. The amount of radioactive glucose retained by the treated cells is determined and compared to that of untreated cells to assess the activity of the test compounds.

A typical assay can be set up as follows:

Sample	buffer	Compound	DMSO	cells	^{14}C-Glucose
blank	447.5		2.5		50
basal	397.5		2.5	50	50
insulin	347.5		2.5	50	50
sample	397.5	2.5		50	50
duplicate	397.5	2.5		50	50

Reaction vials (polyethylene scintillation vials 17mm) containing the appropriate buffer and compounds are set up while the cells are being prepared. 50 µl of insulin at 80 nM, which represent saturation levels, is added to the appropriate samples just prior to addition of the adipocytes. A lower concentration of insulin may also be used in the assay. Dimethyl sulfoxide (DMSO; below 0.5%) is used as a vehicle for the compounds of the invention. Test compounds at 200x, depending on solubility in DMSO (approximately 50 µM), is used. The

adipocytes in 50 Ml aliquots is added to the reaction vials and incubated at 37° C for 30 minutes with gentle shaking. ^{14}C-Glucose is then added to each sample which was incubated for a further 60 minutes at 37°C with shaking.

The cells are separated from the reaction buffer by centrifugation. The amount of glucose taken up by the cells is determined by standard scintillation counting. Thus the cells (in duplicates) can be separated by centrifugation in narrow bore microcentrifuge tubes (5.8 x 47.5 mm, 0.4 ml volume) containing 100 µl of dimethyl silicon fluid SF96/50). Each reaction sample is then stirred to ensure even distribution of cells and 200 µl is transferred to a narrow bore tube. The mixture is centrifuged at 13000 rpm for 10 minutes. After centrifugation, the cells float to the top and are separated at the silicon fluid interface. The top layer is then transferred to a borosilicate vial with 7-10 ml scintillation fluid and counted for approximately 10 minutes. 50 µl of ^{14}C was used a control for the total amount of radioactivity in each reaction.

Cellular Insulin Receptor Activation Assay

This assay is used to provide a consistent method for determination of catalytic insulin receptor activity in intact cells in a 96 well ELISA format. A EY-20mer peptide is used as the IR substrate in vitro for determination of the activation state of IR in an enzyme-linked-immunosorbent-assay (ELISA).

Reagent and suppliers:

1) Corning 96-well ELISA plates (Corning cat.# 25905-96)

2) PBS (Phosphate Buffered Saline)
Formulation: 2.7mM KCL
1.1 mM KH_2P0_4
1.5 mM $MgCl_2$ (anhydrous)
138 mM NaCl
8.1 mM Na_2HP0_4

3) HNTG lysis Buffer
Formulation: 20 mM HEPES buffer pH 7.5

150 mM NaCl
0.2% Triton X-100
10% Glycerol

4) HNTG* Formulation:
1X HNTG
5mM Na_3O_4
2mM NaPPi
5mM EDTA

5) DMEM (Dulbecco's Modified Eagle Medium) with 1X high glucose, L-Glutamine (GibCco Cat# 11965 -050)

6) FBS (Fetal Bovine Serum) (GibCo Cat# 16000-044)

7) L-Glutamine (200mM stock) (GibCo Cat# 11965-050)

8) Growth media: DMEM 10% (heat activated) FBS+ 2mM L-glutamine

9) Starve media: DMEM

10) H25 cells (NIH 3T3c7 cells transfected with a plasmid expressing the human insulin receptor) grown in growth media containing 5% CO_2 at 37° C.

11) Anti insulin receptor antibody (Santa Cruz Biotech) (SC-710 or SC-09).

12) EY-Tide: Biotin linked peptide (sequence biotin EYEYEYEYEYEYEYEYEYEY, MW=3280.4) (Protein chemistry lab SUGEN, Inc.).

12) ABTS solution:
formulation: 100 mM Citric Acid (anhydrous)
250mM Na_2HPO_4, pH 4.0, 0.5 mg/ml ABTS (2,2-azino-bis (3-ethylbenzthiszoline-6-sulfonic acid) (Sigma Cat# A4-888), keep solution in dark at 4° C until ready to use.

14) Hydrogen peroxide 30% : Fisher (cat#H325).

15) ABTS/H_2O_2:
formulation: 15 mL ABTS solution
2 µL H_2O_2

16) Kinase Buffer
formulation: 25 mM HEPES/CL, pH 7.0
150 mM NaCl
0.1% Triton X-100
10 mM $MnCl_2$

17) TBST Buffer (Tris-buffered Saline with Triton X0199)
formulation: 50 mM Tris, pH 7.2
150mM NaCl
0.1% Triton X-100

18) Polypropylene 96-well V bottom plates. (Applied Scientific Cat. # AS-72092)

19) Blocking buffer: 5% powdered non-fat milk in PBS (w/v).

20) ABC kit : Avidin-HRP Developing reagents : Vector Labs (Cat. # PK-4000)

21) DMSO (Dimethylsulfoxide): Sigma (Cat. # D-8418)

22) ATP : Sigma (Cat. # D-8418)

23) 4G10: Phosphotyrosine specific monoclonal antibody (UBI)

24) Insulin, Crystalline, Bovine, Zinc: GibCo cat# 13007-018

Assay Procedure

1) Seed H25 cells in 96 well Tissue culture plate at 15,000 cells well in Growth media.

2) Coat Corning ELISA plate with 0.5 µg/well of anti-insulin receptor antibody in PBS. Final volume per well is 100 µl. Keep plate over night at 4° C.

3) Coat another Corning ELISA plate with 0.5 µg/well of 4G10 antibody in PBS. Final volume per well is 100 µl. Keep plate over night at 4° C.

4) Starve cells 2 hours before assay by removing growth media, washing with PBS and adding 90 µl of starve media.

5) Remove unbound antibody from wells of anti-insulin receptor antibody coated plate by inverting plate to remove liquid. Wash plate 3x with TBST.

6) Block plate with 150 µl blocking buffer per well. Incubate while shaking on a microtiter plate shaker at room temperature for 30 minutes.

7) Dilute compounds/extracts and DMSO controls 1:10 in DMEM on a polypropylene plate.

8) Add diluted compounds/extracts and DMSO controls 1:10 to 96 well cell plate. Incubate plate for 20 minutes in incubator at 37°C and 5% CO_2.

9) After 20 minutes of incubation add 100 µl of 0.2 µM insulin to positive control wells. This results in a final insulin concentration of 0.1 µM per well. Incubate plate for 10 minutes in incubator at 37°C and 5% CO_2.

10) Remove media from cell plate by inverting plate to remove liquid. Wash 1x with PBS, then add 100 µl/well HNTG*. Let plate sit on ice for 5 minutes.

11) Remove blocking buffer and wash the anti-insulin receptor antibody coated ELISA plate 3x times with TBST. Pat the plate on a paper towel to remove excess liquid and bubbles.

12) Use a 12 channel pipetter to scrape the cells from the plate, and homogenize the lysate by repeating aspiration and dispensing. Transfer the lysate to the corresponding wells of the anti-insulin receptor antibody coated ELISA plate. Incubate while shaking on a microtiter plate shaker at room temperature for 1 hour.

13) Remove unbound protein from wells by inverting anti-insulin receptor antibody coated ELISA plate. Wash plate 3x with TBST. Pat the plate on a paper towel to remove excess liquid and bubbles.

14) Prepare kinase buffer solution containing 6 µM peptide and 2 µM ATP. Add 50 µl of solution to each well. Cover ELISA plate with Parafilm and incubate while shaking on a microtiter plate shaker at 4°C overnight (approximately 15-17 hrs.).

15) The next morning block a 4G10-coated plate with 100 µl blocking buffer to each well. Incubate while shaking on a microtiter plate shaker at room temperature for 30 min.

16) Remove anti-insulin receptor antibody coated plate from 4°C cooling medium and add 50 µl of kinase buffer to each well.

17) Wash UB40 coated plate 3X with TBST. Pat the plate on a paper towel to remove excess liquid and bubbles.

18) Transfer 80 µl/well of kinase buffer (containing EY Tide peptide and ATP) from anti-insulin receptor antibody coated plate to the corresponding wells of the 4G10 coated plate. Incubate for 30 minutes while shaking on a microtiter plate shaker at room temperature. Discard the anti insulin receptor antibody coated plate.

19) Right after above step mix up ABC kit reagent in 15 ml of TBST. Vortex and incubate on bench top for 30 minutes.

20) Wash UB40 coated plate 3x times with TBST. Pat the plate on a paper towel to remove excess liquid and bubbles.

21) Add 100 µl/well of pre-mixed ABC reagents to UB40 plate. Incubate for 30 minutes while shaking on a microtiter plate shaker at room temperature.

22) Wash 4G10-coated plate 3x times with TBST. Pat the plate on a paper towel

to remove excess liquid and bubbles.

23) Remove liquid from plate and wash 3x with TBST.

24) Add 100 µl/ well of ABST/H_2O_2 solution. Incubate for 5 minutes while shaking on a microtiter plate shaker. Place ELISA plate in ELISA plate reader and determine absorption at 410 nm, reference at 630 nm.

Results are calculated as an IC50.

CONCLUSION

Thus, it will be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are expected to modulate the activity of protein tyrosine enzymes which mediate cellular signal transduction, in particular, protein tyrosine phosphatase, and therefore are expected to be effective as therapeutic agents against disorders associated with protein tyrosine enzyme related cellular signal transduction.

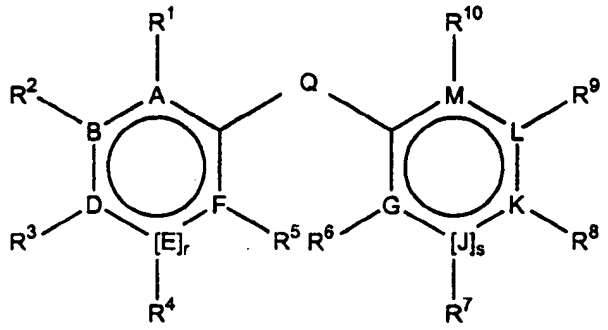
Although certain embodiments and examples have been used to describe the present invention, it will be apparent to those skilled in the art that changes to the embodiments and examples shown may be made without departing from the scope and spirit of this invention.

Other embodiments are within the following claims.

CLAIMS

WHAT IS CLAIMED:

1. A heteroaryl compound having the following chemical structure:



wherein,

r and s are independently 0 or 1; wherein,

when r or s is 1 then A, B, D, E, F, G, J, K, L, and M are independently selected from the group consisting of carbon and nitrogen such that six-member nitrogen heteroaryl rings formed are those known in the chemical arts; it being further understood that when A, B, D, E, F, G, J, K, L or M is nitrogen, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 or R^{10}, respectively, does not exist;

at least one of A, B, D, E or F and at least one of G, J, K, L and M must be nitrogen;

when r or s is 0 then A, B, D, and F or G, K, L and M, respectively, are independently selected from the group consisting of carbon, nitrogen, oxygen and sulfur wherein the juxtaposition of A, B, D and F and of G, K, L and M are limited to those five-member heteroaryl groups known in the chemical arts and it is understood that when A, B, D, F, G, K, L or M is oxygen or sulfur or A, B, D, F, G, K, L or M is nitrogen and that

nitrogen is participating in a heteroaryl ring double bond, R^1, R^2, R^3, R^5, R^6, R^8, R^9 and R^{10} do not exist and there is no bond;

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, alkoxy, aryloxy, thioalkoxy, thioaryloxy, heteroaryloxy, heteroalicycloxy, sulfinyl, sulfonyl, S-sulfonamido, N-Sulfonamido, trihalomethanecarbonyl, trihalomethanesulfonyl, carbonyl, C-carboxy, O-carboxy, C-amido, C-thioamido, N-amido, hydrazino, cyano, nitro, halo, O-carbamyl, N-carbamyl, O-thiocarbamyl, phosphonyl, N-thiocarbamyl, guanyl, guanidino, ureido, amino, trihalomethane- sulfonamido, and $-NR^{11}R^{12}$; wherein,

R^{11} and R^{12} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, carbonyl, C-carboxy, sulfonyl, trihalomethanesulfonyl, trihalomethanecarbonyl and, combined, a five- or six-member heteroalicyclic ring;

when r or s is 0 and A, B, D, F, G, K, L or M, respectively, is a nitrogen atom which is not participating in a heteroaryl ring double bond, then R^1, R^2, R^3, R^5, R^6, R^8, R^9 and R^{10} are selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, trihalomethanecarbonyl, sulfonyl, trihalomethane- sulfonyl, cyano, C-carboxy, O-carboxy, C-amido, C-thioamido and guanyl;

Q is selected from the group consisting of oxygen, sulfur, sulfinyl, sulfonyl and $-NR^{13}$;

wherein,

R^{13} is selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, cyano, trihalomethanecarbonyl, sulfonyl, trihalomethanesulfonyl, C-carboxy, O-carboxy, C-amido, C-thioamido and guanyl;

Any two adjacent R groups may combine to form an additional aryl, cycloalkyl, heteroaryl or

heteroalicyclic ring fused to the ring initially bearing the R groups; and,

physiologically acceptable salts and prodrugs of said compound.

2. The compound, salt or prodrug of claim 1 wherein, Q is selected from the group consisting of sulfur and -NR^{13}.

3. The compound, salt or prodrug of claim 2 wherein, R^{13} is selected from the group consisting of hydrogen and alkyl.

4. The compound, salt or prodrug of claim 1 wherein:

r is 0;

s is 0; and,

Q is sulfur.

5. The compound, salt or prodrug of claim 1 wherein:

r is 0;

s is 0;

Q and S are sulfur;

F is nitrogen; and,

R^2 is nitro.

6. A pharmaceutical composition of said compound, salt or prodrug of claims 1, 2, 3, 4 or 5.

7. A method for the modulation of the activity of a protein tyrosine enzyme

related to cellular signal transduction comprising administering one or more of said compounds of any of claims 1, 2, 3, 4 or 5 to said protein tyrosine enzyme.

8. The method of claim 7 wherein, said protein tyrosine enzyme comprises a protein tyrosine phosphatase.

9. The method of claim 7 wherein, said protein tyrosine enzyme comprises a protein tryrosine kinase.

10. A method for treating a disorder associated with abnormal protein tyrosine enzyme related cellular signal transduction comprising administering to a patient suffering from said disorder a therapeutically effective amount of one or more said compounds, salts or prodrugs of claim 6.

11. The method of claim 10 wherein, said protein tyrosine enzyme is a protein tyrosine phosphatase.

12. The method of claim 10 wherein, said protein tyrosine enzyme is a protein tyrosine kinase.

13. The method of claim 10 wherein said patient is a mammal.

14. The method of claim 13 wherein said mammal is a human.

15. The method of claim 10 wherein said disorder associated with abnormal protein tyrosine enzyme related cellular signal transduction is selected from the group

consisting of glioma, melanoma, Kaposi's sarcoma, hemangioma, ovarian cancer, breast cancer, lung cancer, pancreatic cancer, prostate cancer, colon cancer and epidermoid cancer.

16. The method of claim 10 wherein said disorder associated with abnormal protein tyrosine enzyme related cellular signal transduction comprises diabetes mellitus.

17. The compound of claim 1 wherein, r, s, A, B, D, E, F, G, J, K, L, M, Q, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are selected so as to form a molecular structure set forth in Table 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12333

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2,192,796 A1 (Tang et al.) 08 December, 1996. See the entire document especially, page 7, formula II; page 20, formula III, and lines 1-29; page 21, the entire page; page 22, formula IV.	1-17
X	Database Caplus, on STN, AN-95132727 JACQUES et al., Nitroheterocyclics of chemotheraeutic interest. Synthesis , Polarography and Antiparasitic Activities of Thiazole and Pyridine Analogs. Eur. J. Med. Chem. - Chem. Ther. (Abstract and Figures), vol. 16, No. 3, pages 233-239, 1981. See the entire figures.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 SEPTEMBER 1998	Date of mailing of the international search report 16 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer TAOFIQ A. SOLOLA Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12333

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12333

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/44, 47, 53, 54, 425, 445, 495, 505, 535; C07D 215/02, 237/26, 239/02, 241/36, 249/02, 251/72, 253/10, 261/20, 275/02, 277/20, 285/08, 285/16, 403/14, 411/14, 417/14, 471/02, 487/06

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/222.5, 236.2, 242, 249, 252, 253, 255, 274, 300, 312, 326, 369; 544/8, 122, 180, 239, 240, 253, 256, 257, 354, 366; 546/118, 157, 276, 277, 280; 548/136, 141, 181, 182, 184, 213, 221, 229, 242, 263.2

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/222.5, 236.2, 242, 249, 252, 253, 255, 274, 300, 312, 326, 369; 544/8, 122, 180, 239, 240, 253, 256, 257, 354, 366; 546/118, 157, 276, 277, 280; 548/136, 141, 181, 182, 184, 213, 221, 229, 242, 263.2

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-17, drawn to compounds.
Group II, claims 1-17, drawn to second compounds.
Group III, claims 1-17, drawn to third compounds.
Group IV, claims 1-17, drawn to fourth compounds.
Group V, claims 1-17, drawn to fifth compounds.
Group VI, claims 1-17, drawn to sixth compounds.
Group VII, claims 1-17, drawn to seventh compounds.
Group VIII, claims 1-17, drawn to eight compounds.
Group IX, claims 1-17, drawn to ninth compounds.
Group X, claims 1-17, drawn to tenth compounds.
Group XI, claims 1-17, drawn to eleventh compounds.
Group XII, claims 1-17, drawn to twelfth compounds.
Group XIII, claims 1-17, drawn to thirteenth compounds.
Group XIV, claims 1-17, drawn to fourteenth compounds.
Group XV, claims 1-17, drawn to fifteenth compounds.
Group XVI, claims 1-17, drawn to sixteenth compounds.
Group XVII, claims 1-17, drawn to seventeenth compounds.
Group XVIII, claims 1-17, drawn to eighteenth compounds.

Above groups are based on the specific species disclosed in the specification.

The inventions listed as Groups I-XVIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: all the compounds share -S-, -O-, or -N- bridge which is known in the art.

The special technical feature in group I is that the compounds contain thiodiazole, thioazole or diazole as the only heterocyclics. The special technical feature in group II is that the compounds contain thioazole, thiophene, triazole, tetrazole, benzimidazole, benzothiazole, benzodioxane, diazole, or furan as the only heterocyclics. The special technical feature in group III is that the compounds contain 1,2-oxazole, thiopinyl, triazole and thiophene as the only heterocyclics. The special technical feature in group IV is that the compounds contain 1,3-oxazole, triazole and thiophene as the only heterocyclics.
The special technical feature of group V is that the compounds contain triazole and thiophene as the only heterocyclics.
The special technical feature of group VI is that compounds contain morpholino, diazine, triazole, and thiophene as the

only heterocyclics.

The special technical feature of group VII is that the compounds contain triazine, triazole and thiophene as the only heterocyclics. The special feature of group VIII is that they have thiadiazine and thioazole as the only heterocyclics. The special feature in group IX is that they have 1,4-dioxine, triazole and thiophene as the only heterocyclics. The special technical feature of group X is that they have 1,3-dioxine, thioazole, diazole, benzimidazole and thiophene as the only heterocyclics. The special technical feature of group XI is they have 1,2-dioxine optionally fused with imidazole, triazole or thioazole as the only heterocyclics. The special technical feature is group XII is that they have pyridine, pyridyl, diazole, oxadiazole, triazole or thioazole as the only heterocyclics. The special technical feature of group XIII is that they have qunidyl and thiazole as the only heterocyclics.

The special technical feature of group XIV is that they have benzo-1,3-diazine and thiazole as the only heterocyclics. The special technical feature in group XV is that they have pyridylimidazole and thiazole as the only heterocyclics. The special technical feature of group XVI is that they contain purine, triazole or thiophene as the only heterocyclics. The special technical feature of group XVII is that they have pyrazine fused with pyrimidine, and thioazole as the only heterocyclics. The special technical feature of group XVIII is that they contain quinoxalyl, triazole and thiophene as the only heterocyclics.